STUDIES UPON THE BLACKLEG DISEASE OF THE POTATO, WITH SPECIAL REFERENCE TO THE RELATIONSHIP OF THE CAUSAL ORGANISMS

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STUDIES UPON THE BLACKLEG DISEASE OF THE POTATO, WITH SPECIAL REFERENCE TO THE RELATIONSHIP OF THE CAUSAL ORGANISMS

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HISTORICAL REVIEW

The fact that the potato (*Solanum tuberosum*) is subject to maladies like that under consideration in this paper was noted at a comparatively early date in the literature of bacterial diseases of plants. De Jubainville and Vesque (8)[1] mentioned in 1878 a "cellular-rot" of potatoes, radishes, carrots, and beets as occurring in the soil and in cellars; but bacteria are not mentioned as the cause. Their paper and the one following are reviewed by Smith (32).

In 1879 Reinke and Berthold (30) pointed out quite clearly that a wetrot of potatoes could occur without the presence of any fungi. According to Smith, they apparently did not have any active parasite and did not work with pure cultures. However, they were able to inoculate and cause a decay of healthy tubers by means of the watery fluid taken from diseased potatoes, provided especially favorable conditions were supplied in the line of moisture. They were also able to demonstrate the constant association of bacteria with the wetrotting of the tubers.

Prillieux and Delacroix (28) described a disease of the potato stem from France in 1890 and gave the name "*Bacillus caulivorus*" to the organism which they considered to be the cause. Nothing is said in this paper as to the isolation of the organism and the growth of it in pure cultures, although later Prillieux (27, v. 1, p. 16) credits it with producing a green coloration of certain culture media. Prunet (29), who reviewed the literature on this subject in 1902, called attention to the fact that Laurent (21) had stated in 1899 that *B. caulivorus* was probably nothing other than *B. fluorescens liquefaciens* Flügge, a common soil saprophyte. Pethybridge and Murphy (26), who have more recently written on the subject, state that—

> Later on Delacroix, in dealing with *B. caulivorus*, speaks of it as most probably identical with *Bacillus fluorescens liquefaciens* Flügge, a common saprophytic form, which, he suggests, may perhaps under certain special conditions become parasitic.

It is evident that Prillieux and Delacroix (28) did not secure the organism responsible for the disease. However, there is every reason to believe that they were concerned with a malady similar to that under

[1] Reference is made by number to "Literature cited," pp. 124–126.

consideration, for it was characterized by a decay of the stem beginning at the base and extending upwards.

It is to Smith (31) that we owe our first tnorough study and careful description of a bacterial disease of the potato stem and tuber. However, the disease, caused by *Bacillus solanacearum* Smith, belongs to a distinctly different type than blackleg.

As far as the writer has been able to discover, Frank, in Germany, (13, 14) appears to be the first writer definitely to connect the blackleg disease or "*Schwarzbeinigkeit*" of the potato stem and the accompanying wetrot of the tuber with a bacterial parasite. It is evident that the disease had been known and recognized there for some time, but it had been associated with various fungi. Frank's description of *Schwarzbeinigkeit* is definite and clear, and it agrees very closely with certain, but not all, of the characters of the blackleg as observed by the writer in Maine, Vermont, New York, and in some of the Middle West and Rocky Mountain States.

The organism, to which the specific name "*Micrococcus phytophthorus*" was applied in the second paper, was found to be constantly associated with the disease and capable of producing it when inoculated into healthy stems. It is interesting to note that all other organisms described as causing a similar type of disease are bacilli and are of greater length than the diameter, 0.5μ, which Frank gave for the cocci he described. In this connection, however, it may be mentioned also that Frank stated that for four years material studied by him from different regions invariably showed organisms of the type he described.

In 1901 Delacroix (9, 11) described what appears to be still another type of bacterial disease of the potato stem for which was suggested the name "*brunissure*," or browning. He gave the name "*Bacillus solanicola*" to the causal organism. As in the case of blackleg, the first visible signs of disease are a yellowing of the foliage and cessation of growth, followed by the gradual death and drying up of the plant. The stems die from the base upward, but are described as browned rather than blackened; and cross sections show transparent brown patches, which may extend some distance up the stem. A prominent characteristic is the development of a gummy material and tyloses in the wood vessels of the stems of attacked plants.

It is evident that this disease, like that caused by *Bacillus solanacearum*, is of a type entirely distinct from blackleg and therefore need not be considered further, especially since cultures of the organism are no longer available for comparison. It may be mentioned, however, that Delacroix considers it to be a soil organism, and the chief source of infection is from the soil by means of wounds. Tubers are also attacked, the disease entering from the stem end and causing a browning of the tissues.

In 1902 Van Hall (15) in Holland described a bacterial disease of the potato which was similar to that described in Germany by Frank. He named the causal organism "*Bacillus atrosepticus*." This will be considered more in detail in the discussion of the organisms studied by the writer.

From 1902 to 1906 Appel published a series of several papers (1–7) upon his studies of the *Schwarzbeinigkeit* as he had observed it in Germany. His most complete account of the disease and the description of the organism was published in 1903 (4). He expressed a view at that time that possibly more than one organism might be associated with the disease and suggested that the term "*Schwarzbeinigkeit*" should not be considered as applying to a specific disease but as descriptive of a pathological symptom. He also mentioned that not all organisms studied by him which were able to cause decay of the tuber were capable of producing the stem disease as well. However, he pointed out that the organism causing *Schwarzbeinigkeit*, which he studied, was probably identical with the one described by Frank. This appears to be the most generally accepted view, although Appel found it to be a bacillus. He published a fairly complete account of it and named it "*Bacillus phytophthorus*." Smith (33) has more recently repeated and extended Appel's studies, working apparently with his original culture, and his description of *B. phytophthorus* is the most complete one that we have.

In a general account of potato diseases and the disease resistance of potatoes, Jones (19), in 1905, describes blackleg and summarizes his observations upon its occurrence in Europe. He mentions it as being found in Germany, Holland, Belgium, France, and England.

In 1906 Delacroix (12) reported on the presence of the *Schwarzbeinigkeit* in France and compared the appearance of plants affected with the latter and those showing "*brunissure*." He states that blackleg is primarily a disease of the late spring or early summer, while the malady caused by *Bacillus solanicola*, as a rule, appears in midsummer or early autumn. However, he says, in effect, that the exterior characters are such that one can hardly separate the two diseases by them.

In the same year Johnson (18) stated that he had clear evidence of the existence of *Bacillus phytophthorus* as a general cause of "yellow-blight," blackleg, and potato tuber-rot in Ireland, but Pethybridge and Murphy (26) are quite inclined to doubt his evidence.

Jones (20) records for the first time the occurrence of blackleg in the United States, also in 1906. The disease agreed in every respect with that which he had observed in Europe. This was in Vermont on a farm where he had been studying potato diseases for nearly 20 years and had not previously observed it. The seed tubers used for planting came from Maine.

In 1906 Harrison (17) described a potato disease of the same type which he stated to be of wide distribution in Canada and of much eco-

nomic importance there. He regarded the causal organism as a new and distinct species to which he gave the name "*Bacillus solanisaprus*." His study was the most detailed and complete of any which had appeared up to this time upon an organism associated with blackleg.

The writer came to Maine in 1906, too late in the season to find the disease that year, but in 1907 it was found and its presence recorded (22). Preliminary accounts regarding the nature of the disease, economic importance, distribution, manner of dissemination, and means of control were published in 1909 (23) and 1911 (24).

In 1911 Pethybridge and Murphy (26) published a review of the literature on the subject and described a similar type of disease in Ireland. These workers also considered the organism responsible for the type of malady there to possess sufficient distinctive characters to be considered a separate species, although they felt that it was closely related to *Bacillus phytophthorus*. They named it "*Bacillus melanogenes*."

CHARACTER AND APPEARANCE OF THE DISEASE

Blackleg is, as has already been indicated in the preceding section, a bacterial disease of the stem and tuber of the potato. The various common names which have been applied to it have been given largely as a result of the quite characteristic signs of the disease which are exhibited by the attacked stems. In Germany, where the trouble was first described, it has been known for years as *Schwarzbeinigkeit*, although the term "*Stengelfäule*" has also been applied to it. It would seem from the literature that the former name or a somewhat free translation of it is the one which has been in common use in Holland, Belgium, France, and England. Jones (19, 20) described it under the name "blackleg," the name with which he had become familiar in England and Germany. Smith (33) prefers "basal stemrot." Pethybridge and Murphy (26, p. 9–10) state that—

> Since, however, the term "Blackleg" is one which is already in common use in this country for a disease prevalent among cattle, it seems strongly advisable, in order to avoid confusion, not to use the term "blackleg" for the present disease; and we have, therefore, decided to distinguish it by the name of "Black Stalk-rot."

Were the writer describing this type of disease for the first time, he would endeavor to select a somewhat more appropriate name than blackleg and would prefer black stalkrot or black stemrot to this. However, he has found the objections to the former term to be of more theoretical than real importance.

Blackleg, as the name indicates, is characterized in its typical form by a pronounced blackening of the base of the stem of the affected plant. However, such plants frequently show other visible signs of disease before this character is apparent above ground. They first appear more or less unthrifty and usually undersized. The branches and leaves,

instead of spreading out normally, tend to grow upward, forming a more or less compact top, frequently with the young leaves folded and curled up along the midrib. Later they become lighter green and even yellow, and the whole plant gradually dies. If the disease progresses rapidly, quite a different picture is presented, and the plant may fall over suddenly and wilt with very little previous signs of disease. At first sight the general aspect of the affected plants does not differ from that produced from several other causes which injure or kill the parts at or below the surface of the ground, such as the attacks of fungi or insects, or even mechanical injury at or near the base of the stem. Occasionally, and more frequently some seasons than others, when the plants are attacked before setting tubers and when the progress of the disease is slow, numerous aerial tubers are formed on the stalk at the surface of the ground or in the axils of the leaves above. As is well known, this condition may arise from certain other injuries of the stem as well.

The diagnosis of suspected cases is easily confirmed by pulling up the diseased plants. Stems attacked by blackleg show an inky-black discoloration extending from the base of the stem, where it is attached to the seed piece, up to the surface of the soil, except in the early stages, and very frequently 1, 2, or even 3 inches above the ground. Some seasons, under favorable weather conditions, the disease may with considerable frequency follow up the stem for several inches or even out on the larger branches, destroying the entire stem with great rapidity. This is more likely to occur when the plants are growing in a naturally moist soil and during periods of moist, cloudy weather.

If two or more stalks arise from one seed piece and one develops the disease, the remainder will invariably, sooner or later, succumb to it also. Very frequently during the last of July, or during August in Maine, when a diseased stalk is observed, a careful search will reveal close beside it the dried remains of another which died some time earlier. It is these stalks that are not visibly affected till later in the season which usually show the rapid and complete invasion of the above-ground parts of the stem by the bacteria. However, there is no evidence to indicate that the disease is ever communicated from one stalk to another by traveling down one and up the other. In a large number of inoculations of living potato plants in flowerpots in the greenhouse and in boxes of soil in the field, including all regions of the plant from the petioles of the leaves to parts of the stem below the surface of the soil, the writer has seen little evidence, except in the case of certain inoculations on below-ground parts made by Mr. G. B. Ramsey, the writer's associate, in the summer of 1916, of progress of the disease downward on the stem. It is always upward.

With one exception, the writer has not observed blackleg occurring in patches or localized areas. The attacked plants are scattered promiscuously over the field. The pieces of seed tubers from which such diseased

plants have grown are invariably found to have been attacked by a soft wetrot, or may have been destroyed entirely before the examination is made. At this time and for some weeks thereafter the seed pieces from which surrounding, healthy plants have sprung are entirely sound. If young tubers have formed before the plants are destroyed, the disease frequently passes along the stolons upon which they are produced and infects these also, producing a rapid softrot. This is by no means the invariable rule, as might be inferred from the statements of some writers. However, as will be shown later, it is apparently such cases of infection of the growing tubers which are responsible for the propagation and spread of the disease, either directly or indirectly.

The exception noted above, suggestive of the spread of the disease in the field, occurred in Dover, Me., in 1908, on the same field from which pure cultures of the causal organism were first obtained. There was very little blackleg in the entire field of 20 acres, except on one spot of a few square rods where all of the plants were diseased. It was first noted by the owner near the center of the affected area, from which it gradually spread outward. The season had been excessively wet, and this area coincided with a low, undrained pocket or depression in the field, where water would stand for a few hours after each heavy rainfall.

Sometimes the attacked plants attain a height of only 2 or 3 inches above the surface of the soil, and the fact that many hills are entirely missing on fields which show a high percentage of diseased stems indicates that in such cases either the seed pieces decay before sprouting or the sprouts are killed before they reach the surface. As a rule, however, the plants first begin to show signs of disease when they are 6 or 8 inches high and are growing rapidly. In northern Maine this is about the first of July, although the disease frequently makes its first appearance some time in June. Again, the plants may attain nearly full size before many of them on a field appear to be attacked.

The progress of the disease is markedly influenced by weather conditions. Very moist, cloudy weather during the first few weeks of growth may tend to favor rapid progress, resulting in the early death of the young plants, so that only the dead stalks remain scattered among the healthy plants within a month or six weeks or even a less time after its first appearance. A period of dry weather coming on after the disease is well established below the ground may check its progress but cause the death of the plant at an equally early period on account of its inability to withstand the lessened water supply. Again, conditions between these extremes may prolong the attack well into August. More blackleg is observed in wet than in dry seasons.

Fields which showed quite a percentage of blackleg early in July may give a decidedly different impression to the observer by the middle or last of August. At that time the diseased plants may have entirely disappeared; and on account of the scattered occurrence of the former, the

healthy plants, which have reached practically their full development, obscure the vacant areas, giving the appearance of almost a perfect stand over the entire field.

Soil conditions also are factors which influence outbreaks of blackleg. All other things being equal, the disease is more likely to occur in wet than in dry soil, and is more prevalent when the early part of the growing season is characterized by abundant rainfall.

Different varieties of potatoes show a marked difference in their susceptibility to the disease. This is well shown in the case of the Irish Cobbler and the Green Mountain, which represent by far the greater proportion of the potatoes grown in Maine at the present time. The former is an early variety, largely grown for seed purposes for southern planting, and is highly susceptible. The latter is a late variety which is primarily raised for table use and is seldom severely affected with blackleg. In fact, as long as Maine potato growers planted this variety almost exclusively, blackleg was of minor consequence. Harrison (17) has shown by means of inoculation tests that these differences in ability to resist the attacks of blackleg are exhibited by quite a number of varieties.

It should be understood that the above characterization of the disease is made without reference to the published descriptions of this and similar types of potato-stem disease as they occur elsewhere and is based entirely on personal observations which have been made largely in the State of Maine.

There is a somewhat similar form of potato-stem disease which the writer has seen with some frequency in certain Western States, but which is not common in Maine. It differs from the ordinary type of blackleg in that while the stem is blackened and discolored above ground there is little or no external evidence of disease below ground. In all the cases observed, however, a closer examination has shown that the pith has been destroyed between the base of the stem and the externally diseased area above ground.

A few typical cases of this trouble, which some have been inclined to consider as not identical with blackleg, were seen in Presque Isle, Me., in the summer of 1916. In some instances the disease had spread through the stolons to the young tubers. From one of these tubers Mr. G. B. Ramsey isolated a bacterium which produced typical blackleg when inoculated into young, growing stems.

GEOGRAPHICAL DISTRIBUTION OF THE DISEASE

The fact that blackleg or a very similar appearing disease of the potato stem and tuber has been observed in Germany, France, Belgium, Holland, England, Ireland, Canada, and the United States has been pointed out in the previous section.

From the accounts of Frank (13–14) and Appel (1–7) it is evident that the disease is common, widespread, and often destructive in Ger-

many. Delacroix (12) stated in 1906 that he had not seen it in France until within two years and that it was not widespread at that time. At the same time he pointed out the marked similarity of blackleg to the *brunissure* of the stem previously described by him. The possibility is thus suggested that the malady first described in Germany may be of wider distribution in France than has been reported and that the two have been confused. In the same paper Delacroix says that blackleg exists also in Denmark and Russia.

In writing of the occurrence of blackleg in England, Jones (19, p. 17) stated in 1905 that—

> it is said to be common, though apparently less troublesome than Appel reports it from Germany. Reference to the agricultural papers of England during recent years indicates that the disease is of quite widespread distribution in that country.

Harrison (17), in Canada, reports what is probably a much wider territorial distribution of the type of disease he described than was the case with the previous writers. He states that he had found it throughout the Province of Ontario. Its presence had been reported from Nova Scotia, New Brunswick, and Quebec, and one case had been reported from the Northwest Territory.

In the United States the writer has been collecting information regarding its occurrence during the past nine years. It has been reported in most and doubtless occurs in all of the Atlantic seaboard States from Maine to Texas. It probably occurs also on the Pacific coast, and has been recorded from Ohio, Wisconsin, Minnesota, Colorado, Utah, Montana, and Idaho in the interior. In other words, blackleg has been definitely reported from nearly all the great potato-growing centers or States in this country.

There is reason to believe that Maine was one of the first places in which the disease was introduced into the United States, and in connection with the question of geographical distribution it is of interest to speculate on how this came about. It seems reasonable to believe that it was introduced into Maine from Canada and into the Dominion from England. As will be pointed out in a succeeding section, blackleg is carried by seed tubers. Importation of seed stock into Canada from England would naturally be much more common than into the United States; and the wide distribution of the disease in Canada, reported by Harrison, would indicate that it existed there for some time previous to its entry into Maine. Maine's greatest potato-producing section, where the disease was first discovered, is immediately adjoining the Province of New Brunswick. Many of the potato growers in this region formerly resided on the other side of the boundary line, and with the constant intercourse and traffic between the adjoining sections of the two countries it is readily conceivable that blackleg was imported in this way.

In all probability the disease has been introduced in the same way into other States along the northern border. It was carried to the Southern States by northern seed. There is one authentic instance of the introduction of blackleg into this country directly from England.[1] At Kingston, R. I., in the summer of 1907 five hills of potatoes showing blackleg were found. These potatoes were grown from seed tubers which were obtained from England in the spring of the same year. This was the first and only case of blackleg that had been reported from Rhode Island up to that time.

One interesting and apparent case of importation of the disease from Europe and its transference in this country is as follows: In the summer of 1912 it appeared commonly on a field in Parkman, Me., in a part of the State where the disease was practically unknown, and on a farm where the owner had never observed it before. The field was planted with a variety known as Delmany Challenge, which came from Twin Falls, Idaho. Inquiry showed that this variety had been grown on a farm near Twin Falls for three years, having been shipped there from Carbondale, Colo., where it had been planted for two years. The Carbondale grower imported the original seed tubers from Scotland. The writer has since visited the farms mentioned in Carbondale and Twin Falls and found blackleg present in the potato fields on each.

ECONOMIC ASPECTS OF THE DISEASE

Wherever the type of disease under consideration occurs, those who have described it emphasize its economic importance. Apparently a loss of from 5 to 10 per cent of the plants on affected fields is not an uncommon occurrence in Germany, and much greater losses are reported. Frank (13) reports cases where at least 75 per cent of the plants on a given field were killed from this cause alone. Pethybridge (25) reports the result of experimental trials in which apparently sound tubers taken from an infected crop of the previous season produced 94 per cent of diseased plants.

During the past nine years the writer has had opportunity to make some rather extensive observations on this subject. In Aroostook County, Me., in a fairly continuous area, there are about 50,000 acres of potatoes grown annually. In this region there are few potato fields of less than 10 acres, and usually the acreage of individual farmers runs from 20 to 50, although fields of 60 to 80 are not uncommon and there are those which run to 100 acres or more.

Considerable time was spent in this and other potato-growing sections of the State each year, and during the first five years large areas of potatoes were inspected annually. In many fields only scattered plants have been observed and not infrequently these amount to 1 or 2 per cent,

[1] Reported by Prof. G. E. Adams, of Rhode Island State College, in correspondence with the writer.

5 per cent of diseased plants being considered by the growers as representing a severe attack. However, losses amounting to 10 or 15 per cent or more are by no means unknown; and the writer saw one case in 1911 where 50 per cent of the plants had been killed by blackleg or the potatoes had failed to germinate. It should be mentioned that in this instance the seed tubers apparently had been stored under very adverse conditions and were not in a fit condition to plant. The writer has never seen anything like what Pethybridge has described (25), where apparently sound seed tubers, even if they came from a diseased crop, produced a large percentage of diseased plants. As will be shown in a later section, it has been found that the selection of sound tubers from a diseased crop is an important factor in reducing the amount of blackleg in the following crop.

Much less loss has been observed on high, well-drained soils that were well adapted to growing potatoes than on low, wet, undrained soils. Likewise greater damage has resulted from blackleg in seasons of excessive rainfall than in dry seasons. Much greater losses in Maine apparently have been experienced since the advent of the southern seed-growing industry. As has already been mentioned, certain of the early varieties desired for seed purposes by the growers in the South are more susceptible to the disease. Blackleg also appears to be more destructive in the South than in Maine.

The above discussion has been limited largely to the losses resulting from the failure of the seed tubers to germinate; or if they do germinate, to produce plants which will live to mature a crop. The losses which result from tuber decay either in the field or in storage should also be considered.

Nearly all writers who have discussed blackleg have laid special stress upon the amount of loss which it occasions through destruction of the tubers. Harrison (17) estimates a total loss from rot of from 10 to 75 per cent of the crop in the Province of Ontario. Taking the lower figure and allowing 40 cents per bushel, he states that this would amount to $720,000 in that Province alone.

All of the blackleg-producing organisms described, including those isolated and studied in Maine, are capable of causing a rapid and complete decay of the tubers, especially when they are immature, or immediately following harvesting. There is no doubt that they can and do cause some decay in storage and may be responsible for a considerable amount if storage conditions are unfavorable. The writer is fully aware that the same disease may produce radically different results in different countries under different climatic conditions. However, if he were to base his opinion upon his observations of conditions in the northeastern part of the United States and adjacent portions of Canada during the last 15 or 20 years, he would say that the losses from tuber decay in the field and in storage from this source have been largely overestimated.

It is believed that much of the rot of potato tubers, which in some instances has been attributed to the same organism which produces the blackleg of the stem, is due to entirely different causes. In the Northeastern States epidemics of potato softrot in the field and in storage are quite common, but these invariably follow and are associated with outbreaks of lateblight on the foliage caused by *Phytophthora infestans* De Bary. The decay caused by this fungus, as is well known, is of a dry nature. However, if a tuber lying in a moderately damp soil becomes infected with the fungus, various soil bacteria at once enter into the lesions thus made and a rapid, soft, foul-smelling rot results. Where such decay starts in the field, it is very likely to follow and be very destructive in storage, particularly if the conditions of storage are damp.

Repeated experiments by Jones and his associates in Vermont, Stewart in New York, and Woods and the writer in Maine have demonstrated conclusively that epidemics of such bacterial softrot can be prevented entirely if the parts above ground are kept free from *Phytophthora infestans*, by proper spraying with Bordeaux mixture, until they are ripe or are killed by the frost. Moreover, epidemics of softrot of potato tubers following lateblight were common in New England long before blackleg made its appearance and now occur in sections which are entirely free from blackleg. As has been stated previously, the latter disease has been under observation by the writer to a greater or less extent in Maine each summer since 1907. Two severe epidemics of tuber decay, and other less destructive ones, have been experienced during the same period, but this was entirely controlled where thorough spraying with Bordeaux mixture was practiced. On the other hand, no severe outbreaks of tuber softrot have been observed which were not preceded by lateblight on the foliage, regardless of the amount of blackleg which occurred on the plants in the fields.

It must be conceded, therefore, that ordinarily there is no serious loss from tuber decay from the blackleg organism in Maine. However, it must do some damage of this nature, for there is ample evidence that the infected seed tubers are responsible for the propagation of the disease from year to year. Also it is a natural supposition that conditions which enable the various soil organisms to complete the destruction of the tubers, begun by the lateblight fungus, would also favor decay by the blackleg bacteria. If decay is once started in storage or the tubers are kept under too warm, moist conditions and the latter organisms are present, they certainly would be an important factor in causing the rot. However, the writer has made many attempts to isolate organisms from softrot of potatoes following attacks of lateblight on the foliage; but in no case has he been able to secure a bacterial organism capable of causing the decay independently and alone.

SOURCES OF INFECTION AND MEANS OF DISTRIBUTION

Various other agencies have been suggested as being responsible for the spread of the disease; but a long series of observations have convinced the writer that in Maine the ultimate source of the trouble is, either directly or indirectly, infected seed tubers. A large amount of evidence bearing on this point has been collected during the last nine years, both by observation in Maine and by correspondence with others where Maine potatoes are used for seed. As has already been mentioned, only one case has been observed where the disease appeared to spread in the field; and this is easily explained by peculiar local conditions. Since the organisms, as will be shown later, are killed fairly readily by drying, they are probably incapable of existing for any length of time in a living state on the dry surfaces of potatoes. It is the writer's opinion that they are carried over the winter in decaying, bruised, cracked, or otherwise imperfect seed tubers.

In Maine, potatoes are not stored in covered pits in the ground, as is the case in Ireland and in certain other parts of Europe. They are always kept in dark, cool, well-ventilated cellars or specially constructed potato houses, usually the latter. In these houses considerable attention is paid to ventilation. If possible, no excessive moisture is allowed to develop, and the temperatures are held as low throughout the winter as is compatible with safety to the potatoes. In laboratory experiments the organisms in beef-broth cultures were found alive after a period of 10 months, or until nearly all of the moisture had evaporated from the culture tubes. Temperatures a few degrees above freezing, or in the ice box, have been found to be most favorable for keeping this and similar organisms alive for long periods of time in cultures. Under these conditions the organisms retain their vitality, but multiply at a comparatively slow rate. Therefore it seems probable, if the bacteria are able to enter the interior tissues of the tubers either by natural infection in the field shortly before harvesting, or through wounds or cracks made in harvesting, or even through lesions produced by other parasitic organisms, that, so long as they are supplied with a small amount of moisture, they will remain alive. The low temperatures of storage prevent their rapid multiplication and the resultant decay of the tubers. It is undoubtedly those only slightly affected potatoes which are responsible for the propagation of the disease.

Unfortunately, where blackleg is observed for the first time, it is not always possible to trace the seed tubers to determine whether the disease occurred the season before on the field where they were produced. In all cases where this was possible the answer has been found to be in the affirmative. The only criterion by which to judge matters of this kind is whether or not the characteristic blackening and death of the growing stems occurred. As has already been pointed out, the fact that a large

amount of the previous crop had been lost by decay, whether of the nature of a softrot or not, is not necessarily an indication of the presence of the blackleg disease.

Out of a large number a single case will be cited here as evidence that the disease is first introduced by means of infected seed tubers. In 1907 a 4-acre field on the University farm at Orono, Me., where blackleg had not previously appeared, was planted with seed tubers from several different sources. Along one side three barrels of potatoes, each from a different source, were planted. Quite a percentage of diseased plants were found where one of these barrel lots was used; but a careful search several times during the summer failed to reveal any such on the remainder of the field.

Observations in Maine indicate that under the climatic conditions which exist there infected seed potatoes are the sole source of infection and distribution and that the disease does not live over the winter in the soil. Planting the same field with potatoes two years in succession is quite frequently practiced. Where the disease occurred the first year and sound seed tubers for the second crop were carefully selected and then disinfected with formaldehyde as described in the following section, blackleg was either entirely eliminated or was much reduced, depending upon the thoroughness of the treatment.

On fields which are planted with potatoes the second time in succession there is usually quite a percentage of volunteer plants which spring from tubers which remained in the soil over the winter. These plants are frequently easily recognized by their irregular occurrence on the sides of the rows or between the hills. The writer has never seen such plants affected by blackleg. This observation was quite unexpected, for it seems not unreasonable to suppose that, if the tubers were sufficiently protected from frost to be able to germinate, the bacteria causing the disease might live over in the soil in such infected tubers as well as in storage

CONTROL MEASURES

Laboratory studies showed that the organisms associated with the disease did not form spores, were not resistant to drying, and were readily killed by germicides. This, together with the fact that the disease did not spread from hill to hill in the field and that under Maine climatic conditions decayed, diseased, or otherwise imperfect seed tubers appeared to be the ultimate source of infection, suggested the probability that blackleg might be easily and cheaply controlled or even eliminated from a given farm, field, or locality. The most feasible measures which presented themselves were careful sorting before and at seed cutting, and rigid rejection of all diseased or imperfect seed tubers, especially those which showed any blackened or decayed areas, supplemented as an added precaution by disinfection with formaldehyde or mercuric chlorid.

After certain preliminary experiments and with the realization that no control measures would be of much practical use unless they were effective in the hands of the potato growers themselves, it was decided to ask the cooperation of those interested in the work in order to test these control measures on a large scale under actual field conditions. In 1911, cooperative experiments were conducted by eight practical potato growers in three separate towns. On the eight different farms approximately 300 acres of potatoes were under experiment. Collectively these gentlemen selected and disinfected seed tubers sufficient to plant 142 acres. Formaldehyde solution was used for 88 acres and formaldehyde gas for 54 acres. Detailed statements of the methods used and of the results secured from these experiments have been previously published (24).

The experimental fields were carefully watched by the owners for the appearance of diseased plants. The writer visited some of the farms several times and during the first and third weeks in July made a thorough examination of each experimental field, counting each time the number of diseased plants upon representative areas.

The question might naturally be raised that only two careful counts of diseased plants on each part of the experimental fields during the season would not accurately show the actual amount of blackleg on them, for, as has already been pointed out, the time of the greatest evidence of the disease on a given field may be materially influenced by seasonal climatic conditions. That there may be something to this objection is freely granted; but the writer maintains that it in no way influences the *relative* number of diseased plants on the different plots at the time the counts were made, and this seems to be a fair way of judging the efficiency of the different methods of treatment employed. Moreover, if it were to appear at all on the plots planted at the same time with selected and treated tubers, there seems to be no valid reason why blackleg should not show up equally early in the season as on the check plots planted with unselected and untreated tubers from the same lots or bins. As a matter of fact, the times at which these counts were made were selected because they coincided with the period at which blackleg was most in evidence in surrounding fields that season. Also, as has already been stated, other visits made to the fields at various times during the season and the observations made by the owners of the fields themselves furnish plenty of data to confirm the conclusions derived from the counts of diseased plants.

Taken as a whole, the results of the cooperative experiments were sufficiently clear-cut and conclusive to indicate that the preventive measures outlined are exceedingly efficient if properly carried out. In fact, the uniformity of the results was surprising, since so many individuals, including the men who were employed to cut the seed, were responsible for them. In every case where both carefully selected and treated

seed tubers were used the disease was absolutely eliminated. In two instances this occurred on fields planted the second year in succession, in which considerable blackleg occurred the year before, and also appeared upon the check plots used in the experiment. Also in every case where either disinfection or selection was practiced alone and proper check plots were planted for comparison, the amount of blackleg was materially reduced, except on one field where small inferior tubers were purposely sorted out and planted after first being disinfected with formaldehyde solution.

An analysis of the data furnished by the experiments did not lead to any very definite conclusions as to the relative value of selection of sound, perfect seed potatoes for planting as compared with disinfection with formaldehyde alone. There is no doubt that both are necessary. The writer believes that careful selection of seed tubers and rejection for planting all that are in any way cracked, bruised, discolored, or decayed is absolutely essential, and no amount of disinfection with the present known methods can be relied upon to take the place of it. On the other hand, the formaldehyde treatment appears to be equally essential and must be practiced to supplement selection of seed. No one familiar with bacterial softrots of vegetables would assume or suggest that formaldehyde solution or gas could be relied upon to disinfect entirely or even approximately a tuber the interior of which is partially decayed. The writer has never maintained that this could be done, and it would be unnecessary to refer to it or emphasize the point were it not for the fact that in some instances it has been assumed that he did recommend simply seed disinfection with formaldehyde for the control of the disease.

Unfortunately, the way the experiments worked out there was no opportunity to compare on the same field the relative efficiency of formaldehyde gas and solution. In every case where formaldehyde gas was used the results were less efficient than those where formaldehyde solution was used. For this and other reasons the gas treatment is not recommended for general use.

Numerous other cases have come to the writer's attention where careful selection of sound, healthy seed tubers, followed by disinfection with formaldehyde has either eliminated or largely reduced the amount of blackleg in the resulting crop, but only one of these will be mentioned.

In 1911 the Maine Agricultural Experiment Station purchased two lots of Irish Cobbler potatoes for planting at Highmoor Farm in the central part of the State. These were disinfected with formaldehyde, but no special care was used in selection before planting. In both cases quite a percentage of the resulting plants were attacked by blackleg. The following season the farm superintendent personally saw that the writer's recommendations relative to seed selection and disinfection were rigidly carried out. A clean crop resulted, and since then no blackleg has been observed on this farm.

In 1913 some of the crop raised from these selected and disinfected seed tubers were sent to Director T. C. Johnson, of the Virginia Truck Experiment Station, for planting at Norfolk. Out of over 3,000 plants produced from this seed only two doubtful cases of blackleg were reported. At the same time two lots of seed tubers purchased in the open market gave 6.5 and 7.9 per cent, respectively, of diseased plants, and another lot sent in for testing gave 16.8 per cent.

Undoubtedly much can be gained by uprooting and destroying all blackleg plants in the field as soon as seen, taking care to remove also all tubers which have formed; but the writer has no experimental data bearing on this point. It should be recommended not as a substitute for seed selection and disinfection, but rather to supplement them, for the latter are, of course, equally important in the control of several other diseases which are carried by the tubers.

COMPARATIVE STUDIES OF THE CAUSAL ORGANISMS

Of the various organisms which have been isolated and described in different parts of Europe and in Canada as being the cause of *Schwarzbeinigkeit*, or blackleg of the stem of the potato, and the attendant decay of the tubers, the following are available for study: *Bacillus atrosepticus* Van Hall, *B. phytophthorus* Appel, *B. solanisaprus* Harrison, and *B. melanogenes* Pethybridge and Murphy. As far as could be learned from reading the published articles and descriptions, the different investigators who named the species in question made no comparative, cultural studies in the laboratory, under identical conditions, of the previously described species and the organisms which they themselves had isolated. They based their conclusions with regard to the nonidentity of the species upon the manner in which they found their own organisms to differ from the descriptions previously published.

Therefore, when the writer began his studies of this disease in Maine in 1907, several interesting questions presented themselves. From both a scientific and a practical point of view the most important question was, Is the disease in the United States, especially in Maine, caused by one or more of the named species of blackleg-producing bacteria or by one differing from each of them in certain well-defined characters? In other words, if the previously described organisms were collected together and subjected to the various differential tests usually employed in describing a species of bacteria under exactly identical laboratory conditions, would these differences still hold or would they all prove to be identical with each other and with those found associated with the disease in Maine? The exerience of the writer (16) in a similar study of the very closely related group of bacteria causing a softrot of various vegetables suggested that the last question possibly might be answered in the affirmative. Accordingly, after considerable preliminary study had been made of some 18 different strains of organisms isolated in different parts of

Maine, in order to show that these were probably identical with each other and very closely related to *B. solanisaprus* three of these representing isolations made from diseased plants from widely different parts of the State were selected for detailed study and an attempt was made to obtain all of the available named species for comparison. The names of the organisms thus secured and the sources from which they came are given in a later section.

While the work was in progress, Smith (33) published his report on his studies of *B. phytophthorus*, mentioned below. It should be noted here that while he did not at this time publish in detail on his findings with regard to *B. solanisaprus* he states that: "*Bacillus solanisaprus* Harrison is a very closely related, but not identical organism, causing a similar disease in potatoes." In a letter to the writer, about two weeks before this paper was read, dated December 18, 1909, he stated that he had for three years been making a comparative study of *B. phytophthorus*, *B. solanisaprus*, and *B. solanacearum*. It is evident then that the sentence quoted above was based upon a series of long and careful comparative studies. Therefore the writer is warranted in the beginning in assuming that, while very closely related, there are in the opinion of Smith at least two separate types or species to be considered.

For the purpose of comparison it seems necessary to state as briefly as possible, without sacrificing accuracy of statement, the descriptions of the four previously named species which have been included in the present comparative study with the organisms isolated in Maine by the writer. For convenience, since Smith's description of *B. phytophthorus* (33) is more recent than Appel's, and since Harrison's (17) is the first extended and most complete description of the type of the organism that the writer has found in Maine, these will first be given in detail, followed by a brief statement of the characters by which the descriptions of *B. atrosepticus* and *B. melanogenes* would appear to differentiate the last two named from each of the first and from each other.

DESCRIPTION OF ORGANISMS PREVIOUSLY DESCRIBED

SMITH'S DESCRIPTION OF BACILLUS PHYTOPHTHORUS APPEL [1]

The organism is a non-sporiferous rod, variable in length, usually occurring singly or in pairs, but also forming chains of several individuals; taken from young agar cultures the diameter is about 0.6 to 0.8μ, the length 1.5 to 2.5μ; actively motile by means of peritrichiate flagella; stains readily with ordinary stains, but not by Gram's method; rots potatoes (stems and tubers), cucumbers, tomatoes, etc.; aerobe and facultative anaerobe; organism grayish white on agar and slightly bluish opalescent by transmitted light; surface colonies, on thinly sown +15 agar, 1 mm. or less in diameter in 48 hours at 20° to 23° C., 2 to 3 mm. broad in 4 days; round, smooth, wet-shining, internally reticulated at first, amorphous under 16 mm. and 12 ocular, or with small flocks in the older portion; the buried colonies appear brownish under the microscope, also

[1] Quotation 748 from a paper read at the meeting of the American Phytopathological Society, Boston, Dec. 30 and 31, 1909. (33.)

granular in the center; margin of buried colonies sharply defined; liquefaction of +10 gelatin moderate to rapid; circular white colonies with regular margins on gelatin plates, visible in 18 hours at 30° C., in 26 hours at 21° to 23° C.; on thin-sown gelatin plates colonies grow rapidly and are frequently 2 centimeters in diameter at the end of fourth day at 22° C.; alkaline reaction in gelatin cultures to which litmus has been added; on sterilized potato slow white to yellowish white growth; characteristic rapid white growth and black stain on raw potato (when streaked from agar); grows vigorously and with great rapidity on all neutral and feebly alkaline media; clouds 10 cc. of +15 bouillon in 6 hours at 30° C. and in 24 hours at 13° to 14° C., when inoculated with one 3-mm. loop from a bouillon culture 4 days old at 24° C.; especially good growth on neutralized potato-juice gelatin in which stab-cultures rapidly develop a funnel-shaped liquefaction, but less rapid in my hands than in +10 peptonized beef-gelatin; gradual clouding of salted peptonized beef-bouillon, and production of chains therein and pellicle on undisturbed old cultures; no indol reaction; tolerates in beef-bouillon a considerable amount of sodium chloride (5 per cent) and of sodium hydrate (+50); very active growth in potato-juice with formation of thick pellicle and heavy precipitate; rapid clouding of closed end of fermentation-tubes containing potato-juice, but no production of gas; no growth in Cohn's solution; slight greenish tinge in Fermi's solution on long standing; moderate production of hydrogen sulphide; distinct and persistent nitrite reaction in nitrate bouillon but no gas; grows in peptonized beef-bouillon from −50 to +16 and beyond, also in potato broth acidulated to +46 with citric acid, but no growth when acidulated to +45 with oxalic acid; slow (acid) coagulation of milk with precipitation of the casein; slight reddening and final reduction of litmus in milk; slight production of gas in shake-cultures in some beef-agars; grows in bouillon over chloroform; in streak-cultures it reddens litmus agar decidedly in 48 hours at 20° C. in presence of either dextrose, saccharose, lactose, gelactose or maltose; it blues plain litmus agar decidedly in 48 hours and does not promptly redden the same with addition of dextrine or glycerine; no reddening of litmus in gelatin-cultures; the acid persists on boiling; produces small quantities of gas from innosit (muscle sugar), lactose and mannit; optimum temperature 28° to 30° C.; little growth below 4° to 5° C.; minimum temperature for growth in +15 beef bouillon 1° C. or under; maximum temperature for growth in +15 beef-bouillon about 36° C.; thermal death-point in +15 beef-bouillon 47° C.; 90 per cent destroyed by freezing in bouillon. Appel reports loss of virulence in some of his cultures, but I have not observed any during a period of three years. * * * The following are recommended as quick tests for differential purposes: Very thin sowings on gelatin plates; streaks from agar to sterile raw potato; behavior in blue litmus milk; behavior in nitrate bouillon and in Cohn's solution. The right organism should produce big, round, white colonies promptly on thin sown gelatin plates, and should rot potato tubers promptly.

BACILLUS SOLANISAPRUS HARRISON

The following is a brief of Harrison's original description of this organism (17). No effort has been made to follow the original form, but care has been taken to preserve accuracy of statement:

Bacillus, with slightly rounded ends, variable in size, according to the media and temperature in which it was grown. From freshly infected potato stems and tubers it varies in size from 1.5 to 4μ long and about 0.6 to 0.9μ wide. From beef-peptone-agar cultures 24 hours old at 20° C. the bacteria were short and stout, rather variable in length, ends rather square but rounded, vacuolated with average dimensions 1.2 by 0.6μ. From beef-peptone gelatin +5, 24 hours old at 20° C. the bacilli were short and stout, ends slightly rounded, some vacuolated, dimensions 1 to 2.5 by 0.6 to 0.8 μ. Involution forms, long swollen bent rods in 24 hours at 37° C.; endospores

not seen; flagella, 5 to 15 or more, peritrichously disposed; stained by Van Ermengem's method; organism stained readily with the usual anilin colors; Gram's method was negative, but positive with amyl alcohol; growth in agar abundant, filiform, spreading, elevation raised, luster glistening, opalescent; good persistent growth on potatoes, slightly raised and spreading, dull waxy and pale cream in color which subsequently changed to dirty white; growth in Loeffler's blood serum good and slightly spreading, raised a little with the edges some higher than the center, waxy and pale yellow in color; uniform growth in gelatin stab cultures, filiform, liquefaction begins in 35 days and is not complete in 44 days; strong growth in nutrient broth +15 at 25° C., slower at 20° C.; ring in surface growth; clouding strong and persistent, fluid turbid, fine sediment; prompt coagulation of milk in 48 hours, extrusion of whey in 3 days, a few minute bubbles of gas visible; litmus milk acid, with partial slow reduction; growth in gelatin colonies slow, round to elliptical or egg-shaped in form; elevation, surface colonies flat; edge first entire, later lobate or erose; color faint brown by reflected, and bluish white by transmitted light; agar colonies round to lenticular, surface moist, shiny; elevation flat; edge entire; internal structure finely granular; growth in Fermi's solution similar to that in Uschinsky's medium, but slightly less in amount; copious growth in Uschinsky's solution, ring, sediment, liquid appeared bluish; the best media for long continued growth was found to be beef bouillon and Uschinsky's; in fermentation tubes gas was produced only in bouillon containing mannit and lactose; growth in the closed arm with production of acid in the presence of each of the following: Dextrose, saccharose, lactose, maltose, glycerin, mannit, and levulose; at the end of 10 days the closed arm with mannit, glucose, and lactose was clear, the other tubes remained clouded in the closed arm, but with less turbidity than was observed on the second day of growth; nitrates in nitrate broth were reduced, nitrites present; indol production moderate to feeble; growth in bouillon +16, Fuller's scale, with hydrochloric acid and not at +18; growth in bouillon −16 with NaOH but not at −18; vitality on culture media, long; thermal death point 54° C., the optimum temperature 25° to 28°, slight growth at 37°, none at 42°; maximum temperature for growth 37.5°; minimum temperature for growth about 0°; in poured plates sensitive to sunlight, 90 per cent being killed in 30 minutes and 100 per cent being killed in 50 minutes; pathogenicity proven in the following vegetables: Potato, tomato, Jerusalem artichoke, cucumber, red carrot, white carrot, radish, red beet (sl.), sugar beet (sl.), parsnip, cauliflower, cabbage, celery, mangel-wurzel, Swede turnip, and white turnip; also in living plants of potato, tomato, common red pepper, and slightly in cucumber and physalis.

BACILLUS ATROSEPTICUS VAN HALL

The description of this species was published in Dutch (15) as a part of a dissertation for the doctor's degree. The following is based on a translation of a part of this article, made for the writer by Dr. R. de Zeeuw.

Bacillus occurring in 2-day-old bouillon cultures at 27° C., almost exclusively as single rods, sometimes in pairs; size variable, 0.8 to 1.6μ long by 0.2 to 0.4μ wide, stained with gentian violet; many small zoogloea; very motile at 27° C. in young cultures containing 0.025 per cent of potassium phosphate and 0.25 per cent asparagin; flagella stained from such cultures by Loeffler's method, length 10 to 15μ; Gram's stain decolorized. Growth weak on malt agar and malt gelatin; gelatin liquified, but rapidity is variable, does not take place on unneutralized meat gelatin and is sometimes slight on one weakly alkaline. Strong coagulation of casein in milk. No diastasic action on starch. Gas production weak or absent except where mannit is used as a source of carbon. A medium consisting of "duinwater" (water out of the dunes, filtered through sand and gravel) plus 0.025 per cent of dibasic potassium phos-

phate (K_2HPO_4), 1 per cent peptone, and 3 per cent, respectively, of different carbohydrates gave the following results in three successive trials: Saccharose, 0.1, 0, 0.2; glucose, 0, 0, 0.1; lactose, 0, 0, 0; mannit, 0.4, 0.6, 0.3; glycerin, 0, 0, 0; galactose not changed; nitrates reduced to nitrites; reduction of methylene blue weak; sodium selenite reduced very rapidly; indol production not observed. Growth in bouillon acidulated till the reaction is 0.5 per cent normal with citric and malic acids does not stop growth, but 1 per cent entirely prevents it. Thermal death point for a 24-hour-old culture between 51° and 52° C. Optimum temperature not accurately determined, but strong growth takes place at 27° C. Readily killed by drying.[1] Pathogenicity to potato stems and tubers somewhat variable, but apparently imperfectly tested.

BACILLUS MELANOGENES PETHYBRIDGE AND MURPHY

Pethybridge and Murphy described their organism in considerable detail (26), but it is sufficient for our present purpose to simply state wherein they found it to disagree with published descriptions of previously described organisms. They stated that it was larger in size than *B. atrosepticus* (0.7 to 0.9 by 1.3 to 1.8μ) and instead of occurring chiefly singly was found more frequently in pairs, also that its action upon milk appeared to be different. As contrasted with Harrison's description of *B. solanisaprus*, it possessed less flagella, formed gas in glucose and cane sugar, did not form a distinct ring on the surface of potato juice, and did not produce a raised, creamy white growth on cooked potato. They stated that their organism showed marked resemblance to *B. phytophthorus* and that they were strongly tempted to regard it as only a variety of the latter. However, it did not produce a pellicle on Appel's sterile potato juice, and in nitrate broth it produced a small quantity of gas. In milk it caused the separation of the curd as a not very compact mass and produced a distinct acidity in a comparatively short time. According to Appel's description, *B. phytophthorus* produces a strong pellicle on sterile potato juice, apparently produces no gas in nitrate broth, and causes milk to change only on long standing, forming then a compact cylinder of precipitated curd, and giving a reaction which is amphoteric to litmus.

SOURCES OF THE CULTURES USED

Blackleg was first observed by the writer in Maine at Sherman, on July 30, 1907. It was seen later at Orono and Dover; but in all cases the disease was in its last stages, and attempts to isolate the causal organism that season resulted in failure.

In 1908 diseased plants were obtained from Dover, Piscataquis County, on July 24, which showed the affected tissues filled with actively motile bacteria. Several subcultures were made from plates poured from these stems and all proved to be nonpathogenic.

At the same time the diseased portions of the stalks were mashed up with distilled water in a mortar. Healthy potato tubers, bearing shoots

[1] The author also presents considerable data relative to experiments conducted to determine the available sources of nitrogen and carbon, using a considerable number of different substances, apparently added to the culture medium singly or in combination.

2 or 3 inches long, were thoroughly moistened with this watery extract of the diseased tissues and at once planted in boxes of soil which had previously been thoroughly wet down. A part of the remaining watery extract was then poured over each box. These artificially infected tubers put up shoots very rapidly and on August 15, 22 days after the planting, one stalk began to show the characteristic signs of the disease. On the 18th two others were also plainly affected. Examination showed only motile, rod-shaped organisms in the diseased tissues. Poured plates were made from these stems and 9 subcultures were obtained, all of which proved to be pathogenic to potato tubers and stems, producing typical blackleg when inoculated into the latter. Some preliminary work, especially in the line of fermentation studies, was done with all of these, but one strain, designated as "IIIA," was selected for detailed study.

On August 12, 1908, a potato stem about 18 inches long was received from the University Farm, Orono, Penobscot County, which showed a soft, colorless decay which apparently was progressing very rapidly. The general appearance was decidedly different from the picture presented in typical cases of blackleg. The stem was affected nearly its entire length and there were no signs of blackening. The disease was confined almost entirely to the parenchyma cells of the pith within the vascular ring. Apparently the water conducting system had not been materially affected, as there was no yellowing or other abnormalities of the foliage and very little signs of wilting, although one or two other stalks in the same hill had begun to fall over.

The affected portion had been reduced to a pulpy, watery mass; apparently the middle lamella had been destroyed between the cells which latter had collapsed into irregular, shapeless masses. The liquid between the cells was filled with large numbers of motile bacteria. From plates made from this stem 5 subcultures were obtained, all of which proved to be pathogenic to potato tubers and stems. However, in all inoculation tests extending over a period of eight years, typical cases of blackleg were produced, differing materially from the appearance of the original stem. One strain, marked "SE," was studied as a representative of this type.

In August, 1910, another series of four cultures, of which "IIP" was selected as a representative, were isolated from a typical blackleg stem received from Presque Isle, Aroostook County.

Thus, it will be seen that the organisms which were used for the detailed studies were obtained from somewhat widely separated localities, representing two typical cases of blackleg and a third which looked like a radically different type of stem disease, characterized by a more rapid and much more extensive decay of the stem without the development of any blackening or discoloration. As will be seen later, these proved to be, except for slight variations in size, all of the same type as *B. solanisaprus* which was not unexpected in view of the fact that Harrison has found this organism of wide distribution in Canada and there is considerable reason

to believe that the disease was introduced into Maine from Canada. It should be noted, however, that Smith (33) reports having isolated *B. phytophthorus*, which he considers to be closely related to but not identical with *B. solanisaprus*, from potatoes grown in Maine.

At the beginning of these comparative studies in 1908 an attempt was made to collect cultures of all the available organisms which had been described as causing a similar type of disease upon potato stems and tubers.

So far as could be learned, *B. atrosepticus* Van Hall was the earliest described species available. A culture so named was finally obtained from Král's Bacteriologisches Laboratorium, Prague, Austria.

Difficulty was experienced in obtaining cultures of *B. phytophthorus*. The cultures were requested of Dr. E. F. Smith, of the United States Department of Agriculture, and of Dr. O. Appel, of the Biologische-Anstalt, Dahlem. Dr. Smith, while expressing a willingness to pass along the culture, stated that he preferred not to part with it till he had published on the subject. Two cultures bearing this name were received from Dr. Appel. These were apparently identical. Neither of them proved to be pathogenic, either to potato tubers or stems, on repeated inoculations. Apparently Dr. Appel had lost his original culture, from which, presumably, Dr. Smith's strain was obtained, and writing under date of February 14, 1910, he stated that it had been necessary to make a fresh isolation of the organism before he could send it. The writer again wrote to Berlin in the summer of 1911, and a second culture was sent him. This reached the writer in February, 1912, by hand of Dr. W. A. Orton, of the United States Department of Agriculture, who kindly consented to bring it from Germany. Accompanying the culture was the following statement:

B. phytophthorus is isolated from potato tubers by Dr. Schuster, 1911, at Dahlem, Berlin, whose paper on rot of potato tubers is going to be published.

This culture was also found to be nonpathogenic to potato stems and tubers, and, as will be shown later, showed certain well-marked differences in its behavior on culture media when grown side by side with the culture received earlier from Dr. Appel under the same name. It should be remarked here that Dr. Appel recorded the fact that with him some cultures of *B. phytophthorus* showed a less virulence after growing for some time on artificial media. On the other hand, Dr. Smith (33, p. 749) states: "I have not observed any during a period of three years." None of the other organisms studied by the writer have shown any loss of virulence on long-continued cultures, stock cultures being carried either in potato broth or beef-extract bouillon.

My request to Dr. Harrison for a culture of *B. solanisaprus* was referred to Prof. S. F. Edwards, then of the Ontario Agricultural College, who furnished me with a virulent culture in March, 1909.

B. melanogenes was received from Dr. Pethybridge in 1911. This has also shown a good degree of virulence and like *B. solanisaprus* has repeatedly produced on inoculation from pure cultures a rapid and complete softrot of potato tubers and a typical decay of the stem resembling in every particular the blackleg as it occurs in the field.

METHODS USED

In the present investigation the writer has endeavored to follow as closely as possible the procedures outlined by the Committee on Standard Methods of Water Analyses [1] and those recommended by Smith (32, v. 1).

For what seemed good and sufficient reasons, certain deviations were made from the procedures recommended above; but wherever this has been done, mention has been made of the fact and each named organism or cultural strain has been treated exactly like all others. The most important change has been the substitution of Liebig's extract of meat for infusion of lean beef as a basis for ordinary bouillon, gelatin, agar, and bouillon containing carbohydrates for fermentation purposes.

The reasons for this are that the meat extract is a standard product, compounded on a large scale by a reliable concern, and can be purchased the world over, while, on the other hand, no two lots of lean beef obtained from the market have exactly the same composition with regard to amount of fat or fiber present, or decomposition products developed from the different periods of storage before use. The chief objection to the use of lean beef as a basis of fermentation broths is that it is first necessary to remove the muscle sugar from the meat infusion by inoculation with *Bacillus coli*. Consequently the test for fermentation which follows is made, not in a culture medium most favorable to the growth of the organism to be tested, but in one containing the various metabolic by-products of another. Each lot of meat-extract bouillon before being used for fermentation purposes was first tested for fermentable carbohydrates by filling a fermentation tube with it and inoculating it with *B. coli*. In no case was any gas produced. Moreover, in many tests of bouillon made from Liebig's extract during the last 15 years, in only one instance did any gas develop and then only a small bubble in the end of a fermentation tube, the closed arm of which contained approximately 25 c. c. of medium.

However, a careful comparison was made as to the rate of development and vigor of growth of the various organisms in bouillon made from an infusion of lean beef, and in that made from Liebig's extract. In ordinary bouillon no difference was observed between the two in this respect. In fermentation broth made from the meat extract a better

[1] Report of Committee on Standard Methods of Water Analysis to the laboratory section of the American Public Health Association, presented at the Havana meeting, Jan. 9, 1905. 141 p. Chicago, Ill., 1905. Reprinted from Jour. Infect. Diseases, Suppl. 1, 1905.

growth and more constant results were obtained than with that made from lean beef and freed from fermentable carbohydrates by use of *B. coli*.

All chemicals employed in making media or testing reactions were either chemically pure or were of the highest purity obtainable. In addition to Liebig's extract, Witte's peptone, "Gold label" gelatin or Nelson's photographic gelatin No. 1 were used, the latter entirely during the last of the work. For bouillon 5 gm. of Liebig's meat extract and 10 gm. of Witte's peptone were used for each liter of distilled water. To this were added 15 gm. of agar shreds or flour, or 100 gm. of gelatin if a solid medium was desired. No sodium chlorid other than that contained in the meat extract was added. All media unless otherwise specified were made neutral to phenolphthalein. Distilled water was used for all culture media and Grübler's dry stains formed the basis for all staining liquids.

Tubes of ordinary bouillon and agar were sterilized by heating once in an autoclave for 15 minutes at from 3 to 6 or 7 pounds' pressure. All vegetable media or media made from vegetables or containing sugars were sterilized by flowing steam, for tubes, 15 to 20 minutes at from 99° to 100° C. on each of three consecutive days. In the earlier part of the work gelatin was also sterilized by the discontinuous steaming method. Later it was found that it was less likely to become liquid at ordinary room temperatures if the tubes were sterilized by one exposure in the autoclave to steam under 5 to 7 pounds' pressure for 15 minutes.

At the beginning of the comparative work with the assembled cultures each was transferred to a fresh potato-broth culture every 24 hours for several successive days and then gelatin plates were poured from the last potato-broth culture. From these plates transfers were made from single colonies to tubes of sterile bouillon to furnish fresh subcultures with which to work, after proving that the subcultures thus obtained were pathogenic. This was to insure that the cultures were in as near a uniform condition of vigor as possible before beginning to work. Attempts were made to revive in this way the pathogenicity of the two strains which were received as *B. phytophthorus*, but without success. A separate series of stock cultures of each strain which had not been put through this revivifying process were kept in reserve.

All transfers to media to test cultural features were made from young broth cultures about 48 hours old, when the cloudiness had reached its maximum density. For stab cultures to agar and gelatin a straight platinum needle of approximately 0.5 mm. in diameter was used. All transfers to liquid media were made with a 2-mm. loop of the same sized wire. Unless otherwise specified the cultures were incubated at 20° C. In making records of the detailed features of the morphology, cultural, physical, and biochemical features, etc., of the various organisms the descriptive chart or card adopted by the Society of American Bacteriologists in December, 1907, was used as a model and guide.

DETAILS OF COMPARATIVE STUDIES

MORPHOLOGY

B. atrosepticus, *B. solanisaprus*, *B. melanogenes*, and the three organisms studied which were isolated from Maine potato stems were found to agree in all essential morphological characters except for certain variations in size. They are motile, rod-shaped organisms with peritrichiate flagella, approximately three times as long as broad, occurring singly and in pairs, and in unstained preparations from young cultures often showing chains of several individuals. Frequently, however, rather thick rods with very little difference in the two dimensions were observed, more especially in preparations made from the decayed tissues of inoculated tubers. The organism obtained from Dr. Appel as *B. phytophthorus*, while agreeing in other morphological characters, was distinctly smaller in size and the ratio between length and width was decidedly less. No evidence of spore formation was obtained. Involution forms were not observed, although Harrison (17) reports them for *B. solanisaprus* when grown at 37° C. In this connection it may be mentioned, as is stated later, that at this temperature the writer has been unable to secure visible growth of any of the pathogenic strains either on agar slants or in tubes of beef broth.

For making measurements all of the different strains studied, except that obtained from Dr. Schuster as *B. phytophthorus* (which was a nonpathogenic organism plainly of an entirely different type), were stained with aqueous gentian violet, anilin water gentian violet, aqueous methylene blue, alkaline methylene blue, aqueous fuchsin, and carbol fuchsin. These preparations were made from agar slants 36 to 48 hours old. Flagella were stained by a modification of the Pitfield method.[1]

The results of these measurements are given in tabular form below. Table I gives for each organism the extremes in length and breadth and the average size obtained as shown by all the stains collectively. The second presents the same data for each organism for each separate stain used.

TABLE I.—*Extreme and average measurements (in microns) of each organism as obtained from using six different stains*

Organism.	Width.	Length.	Average size.
B. atrosepticus	0.4 to 0.8	1.3 to 2.5	0.5 by 1.8
B. solanisaprus	.4 to .7	1.0 to 2.2	.5 by 1.4
B. melanogenes	.4 to .8	1.2 to 2.4	.5 by 1.7
IIIA	.5 to 1.1	1.3 to 3.3	.7 by 2
SE	.4 to .8	1.3 to 2.5	.6 by 2
IIP	.4 to .8	1.3 to 2.9	.6 by 1.9
B. phytophthorus from Appel	.3 to .7	.5 to 1.6	.5 by .9

[1] The writer wishes to acknowledge the aid rendered by Messrs. M. Shapovalov and A. Strauss in assisting in making many of these preparations, particularly the laborious task performed by the latter in making the large number of measurements of stained organisms, which is the basis of the data here presented.

TABLE II.—*Measurements (in microns) of each organism arranged according to method of staining*

AQUEOUS GENTIAN VIOLET

Organism.	Width.	Length.	Average size.
B. atrosepticus	0.5 to 0.7	1.6 to 2.5	0.6 by 2
B. solanisaprus	.4 to .7	1 to 1.9	.5 by 1.3
B. melanogenes	.4 to .7	1.3 to 2	.5 by 1.6
IIIA	.5 to .7	1.6 to 2.8	.6 by 2.1
SE	.5 to .8	1.6 to 2.5	.6 by 2
IIP	.4 to .7	1.5 to 2.6	.6 by 1.9
B. phytophthorus from Appel	.3 to .6	.6 to 1.4	.5 by .9

ANILIN WATER GENTIAN VIOLET

B. atrosepticus	0.4 to 0.7	1.3 to 2	0.5 by 1.6
B. solanisaprus	.4 to .5	1.0 to 1.6	.5 by 1.2
B. melanogenes	.4 to .7	1.3 to 2.1	.5 by 1.7
IIIA	.5 to .8	1.5 to 2.5	.6 by 2
SE	.5 to .8	1.6 to 2.5	.6 by 2
IIP	.5 to .7	1.4 to 2.9	.6 by 1.9
B. phytophthorus from Appel	.4 to .6	.5 to 1.6	.5 by 1

AQUEOUS METHYLENE BLUE

B. atrosepticus	0.4 to 0.8	1.3 to 2.3	0.5 by 1.8
B. solanisaprus	.4 to .6	1 to 1.9	.5 by 1.4
B. melanogenes	.4 to .7	1.3 to 2	.5 by 1.7
IIIA	.5 to 1	1.7 to 3.3	.6 by 2.3
SE	.5 to .8	1.6 to 2.5	.7 by 2.1
IIP	.4 to .8	1.6 to 2.9	.6 by 2
B. phytophthorus from Appel	.3 to .6	.7 to 1.3	.5 by .9

ALKALINE METHYLENE BLUE

B. atrosepticus	0.4 to 0.7	1.4 to 2.3	0.5 by 1.8
B. solanisaprus	.4 to .7	1 to 2.2	.5 by 1.5
B. melanogenes	.4 to .8	1.3 to 2.4	.5 by 1.9
IIIA	.5 to 1	1.7 to 2.9	.8 by 2.2
SE	.5 to .8	1.5 to 2.5	.6 by 2
IIP	.4 to .8	1.5 to 2.8	.6 by 2
B. phytophthorus from Appel	.4 to .7	.7 to 1.4	.5 by .9

AQUEOUS FUCHSIN

B. atrosepticus	0.4 to 0.7	1.3 to 2.3	0.5 by 1.8
B. solanisaprus	.4 to .7	1 to 2	.5 by 1.4
B. melanogenes	.4 to .7	1.3 to 2	.5 by 1.6
IIIA	.5 to .8	1.6 to 2.5	.7 by 2
SE	.4 to .7	1.5 to 2.3	.6 by 1.9
IIP	.4 to .7	1.3 to 2.5	.5 by 1.8
B. phytophthorus from Appel	.3 to .6	.5 to 1.3	.5 by .9

TABLE II.—*Measurements (in microns) of each organism arranged according to method of staining*—Continued

CARBOL FUCHSIN

Organism.	Width.	Length.	Average size.
B. atrosepticus	0.4 to 0.7	1.4 to 2.1	0.5 by 1.7
B. solanisaprus	.4 to .6	1.0 to 1.9	.5 by 1.3
B. melanogenes	.4 to .7	1.2 to 2	.5 by 1.6
IIIA	.5 to .8	1.3 to 2.5	.7 by 1.8
SE	.4 to .7	1.3 to 2.1	.5 by 1.6
IIP	.4 to .7	1.3 to 2.1	.5 by 1.7
B. phytophthorus from Appel	.4 to .6	.6 to 1.2	.5 by .9

If the strain known as *B. phytophthorus* from Appel be omitted, it will be seen that, while there are minor variations with different stains, the three organisms from Maine run a little larger than those previously named and described. Of these, IIIA is slightly larger than the other two. Of the remaining three, while *B. solanisaprus* is plainly somewhat smaller, on the average this difference is very slight. Taken as a whole it seems difficult to separate the six different strains on the basis of the morphological characters. It is true that the measurements given differ somewhat from those previously recorded for the described organisms. The writer is not in position to say which are the more accurate, but this does not appear to him to be the important point under consideration. What is given here is the relative measurements from stained preparations of the different organisms, made under as nearly uniform conditions as possible, and measured by the same individual, using the same apparatus for all.

CULTURAL FEATURES

AGAR STROKES.—On agar slants the growth characters of *B. atrosepticus*, *B. solanisaprus*, *B. melanogenes*, IIIA, SE, and IIP were identical in every respect except as noted below. The growth was moderate, filiform, with a slight tendency to produce pseudopod-like branches on the surface, if the latter was slightly moist, or such branches might grow up from the bottom of the slant from the condensation water. Elevation flat to slightly raised, luster glistening, topography smooth. Slightly but distinctly bluish opalescent to transmitted light (a smear on the surface of slants with a loop would produce a decidedly opalescent growth). Color pearly white, odor absent, consistency butyrous, slightly viscid in the case of *B. solanisaprus* and *B. melanogenes*. No discoloration of the medium was observed in any of the cultures of the organisms named above.

B. phytophthorus from Appel differed from the above in that the growth, though moderate, was spreading and very finely plumose, giving a characteristic appearance under a low-powered hand lens. *B. phytophthorus*

from Schuster also showed characters on agar slants which differentiated it from all of the other cultures, including the one just mentioned. The growth while filiform at first was more abundant, and later it spread out in a broad band one-third to one-half the width of the slant. It was thicker, somewhat convex and yellowish, and not opalescent. It very soon imparted a very distinct yellowish-brown color to the substratum.

AGAR STABS.—The first six organisms mentioned in the above section produced the same appearance on agar stabs. The growth was fairly uniform but slightly best at the top, although perhaps not more than would result from the heavier inoculation of the upper layers of the medium. The entire surface was covered in from a few days to about a week, depending upon the amount of free moisture present. The line of puncture was filiform to very slightly papillate.

The two organisms carried under the name "*B. phytophthorus*" grew decidedly best at the top, and the one from Schuster caused a distinct browning of the medium, beginning at the surface and diffusing downward.

COOKED POTATO CYLINDERS.—The character of the growth on the cooked potato has been a prominent one among those given by different authors as differentiating from the others in the group the organisms which they have described, consequently considerable attention was given to this topic.

For the work potatoes were chosen which were either freshly dug or had been in storage only a few weeks. In all cases the tests were made on slanted cylinders of sterilized potato in test tubes containing a small amount of distilled water. The tubes were inoculated as soon as sterility was proven, by making a single stroke with a straight needle along the center of the slant.

Inoculations made to tubes of this kind gave practically identical growth characters for *B. atrosepticus*, *B. solanisaprus*, *B. melanogenes*, IIIA, SE, and IIP, which are as follows:

At 20° C. little or no growth apparent in 24 hours; needle growth plainly evident in 48 hours, and if the slant is rather moist, the growth may have spread out by this time to cover a considerable portion of the lower part; in three days, growth moderate to abundant, filiform to spreading, depending upon the amount of water in the substratum; one week, growth abundant and spreading, usually covering the entire surface of the slant. Elevation first convex, then slightly raised to flat; luster glistening; topography smooth to slightly rugose; color yellowish white, resembling pus in appearance, later dirty white; odor not apparent at first, but later slight odor of decayed potatoes; consistency butyrous, with a slight tendency to viscidity in the case of *B. solanisaprus* and *B. melanogenes*. Medium at first slightly grayed, and at the end of a week plainly grayed.

The organism received from Appel as *B. phytophthorus* produced no apparent growth upon potato cylinders, but the liquid below was clouded, and the substratum grayed or slightly browned.

The organism received from Schuster as *B. phytophthorus* upon cooked potato agreed with the detailed description of the group given above, except that in the earlier stages the growth was more abundant, and at all times it had a distinct yellow color, especially on the more moist portions of the slant. This agreed quite closely with the "honey-yellow" color which Appel originally described for *B. phytophthorus*. The medium was considerably grayed and had a brownish tinge.

GELATIN STABS.—The behavior of the various named species with reference to liquefaction in gelatin stab cultures had been used also as a differential character. While all are reported as liquefiers, Harrison (17), who did a large amount of work with *B. solanisaprus*, reports this organism as very slow in this respect, liquefaction not beginning until 35 days, and not complete in 44 days. The others are reported to liquefy gelatin much more rapidly, although van Hall (15) stated that with *B. atrosepticus* liquefaction was variable.

Therefore in the present studies particular attention was paid to the behavior of the various organisms in gelatin stabs. With regard to *B. atrosepticus*, *B. solanisaprus*, *B. melanogenes*, IIIA, SE, and IIP, the behavior was somewhat erratic on this medium, especially when the "gold label" gelatin was used; sometimes one would liquefy the medium more slowly, and sometimes another. For example, in one case with *B. solanisaprus* liquefaction began on the third day, and was not complete in 60 days. In another series the same organism, incubated at the same temperature, 20° C., showed the beginning of liquefaction in less than 24 hours, which was complete in 7 days, or the shortest time observed for any of the series. After Nelson's photographic gelatin, No. 1, was adopted, the results were much more uniform. So close were these at times that in a series of duplicate or triplicate cultures of the 6 organisms named it would be impossible to pick out those which were of the same name, except for the labels on the tubes.

Therefore the final conclusion was that *B. atrosepticus*, *B. solanisaprus*, *B. melanogenes*, and the three organisms from Maine present no well-defined differential characters upon gelatin stab which would separate them from each other. The observed characters of the six on gelatin may be briefly summarized as follows: A needle growth was apparent in 12 hours at 20° to 22° C. Liquefaction as a rule begins in from 18 to 24 hours, and may not begin until the third day. It is usually complete in about 10 days, but may be delayed for some time longer. Usually liquefaction is infundibuliform to slightly napiform and later may assume a saccate appearance. Occasionally liquefaction starts off rapidly at the surface, giving a distinct crateriform appearance, which is quite likely to

change to stratiform, and progresses very slowly from that time on. These variations were common to all of the pathogens and were not confined to any one of the series. No change of color or fluorescence was observed. The liquefied medium is quite turbid at first, and a copious flocculent or granular, whitish deposit settles out into the bottom of the funnel.

Of the two cultures received as *B. phytophthorus*, that from Appel did not liquefy gelatin and that from Schuster produced first a crateriform and then a stratiform liquefaction of only the upper portion of the medium, which was browned.

NUTRIENT BROTH.—In neutral nutrient broth (10 c. c. inoculated with a 2-mm. loop of young broth culture) the clouding in the case of *B. atrosepticus*, *B. solanisaprus*, *B. melanogenes*, IIIA, SE, and IIP is very rapid. It is apparent in 13 to 16 hours at 20° C., varying somewhat with different strains, very evident in 18 hours, and is quite marked in from 24 to 48 hours. Later moderate to strong, persistent, the fluid being frequently turbid in old cultures. Usually a very slight ring may be observed in young cultures, and occasionally a very slight, granular pellicle is formed during the first few days of growth which readily breaks up and falls to the bottom if the tube is handled or even slightly jarred. The sediment is compact, scant, granular, and dirty white. No odor was observed and no discoloration of the medium. As a rule, a very slight viscidity in both sediment and liquid was noted in the case of *B. solanisaprus* and *B. melanogenes*. Otherwise the six above-named organisms were exactly identical in respect to observed characters upon bouillon.

The organism received from Appel as *B. phytophthorus* differed from the above in that with it the sediment is very adhesive. While it was not strictly viscid it resisted pulling apart with the needle and then appeared very stringy. There was no evidence of viscidity of the medium itself in cultures of this organism. The organism received from Schuster as *B. phytophthorus* differed from all the others in that in old cultures the medium was distinctly browned. While the sediment was dirty white it was rather more abundant, and appeared more stringy and viscid than was the case of the other culture carried under this name.

GROWTH IN POTATO BROTH.—The potato broth used was made as follows: Sound, fresh, recently harvested potato tubers were used and after peeling and washing, 500 gm. were grated directly into 1000 c. c. of distilled water. This potato pulp and water was heated for one hour at a temperature of 55° C. and filtered first through cotton and then through filter paper to remove the starch and pulp. The filtrate was then placed in the steamer for 40 minutes at 99° to 100° C., and again filtered through paper. Titration showed the natural acidity to be equivalent to +10 Fuller's scale and this was not changed. The medium was then tubed and sterilized by fractional steaming—15 minutes at 99° to 100° C.

on each of three consecutive days. It gave a faint blue color on the addition of iodin solution, showing the presence of a small amount of starch.

In ordinary culture tubes the appearance produced by *B. atrosepticus*, *B. solanisaprus*, *B. melanogenes*, IIIA, SE, and IIP was absolutely identical, except that the last named produced somewhat more turbidity with less cloudiness. The characters shown were essentially those recorded for beef bouillon except that the growth was somewhat more vigorous and clouding appeared more rapidly, with a greater tendency to produce a ring or pellicle on the surface. This latter was of a granular nature and was less easily broken up than was the case with the pellicle formed on beef bouillon.

In fermentation tubes filled with potato broth there appeared a thorough, uniform clouding of both bulb and closed arm in from 18 to 24 hours, but no gas of any kind was formed.

With the cultures received as *B. phytophthorus* from Appel and as *B. phytophthorus* from Schuster the growth in ordinary culture tubes was the same as in beef bouillon except that with them it was also somewhat more vigorous. In fermentation tubes containing the potato broth *B. phytophthorus* from Appel showed a marked contrast in the appearance of the same organism in fermentation tubes of ordinary beef bouillon containing carbohydrates. With the latter it was never observed to produce any visible clouding of the closed arm of the tube, while in the tubes of potato broth there was a faint clouding of the closed arm with a very perceptible growth on the third day. In the case of *B. phytophthorus* from Schuster the characters were not essentially different from those observed with ordinary bouillon plus carbohydrates. There was a rapid and heavy clouding of the bulb, followed by a slow clouding of the closed arm.

After the cultures had grown for 10 days, the broth was tested with iodin solution, but in no case did there seem to be any diminution of the amount of starch present.

Milk.—Very little difference could be observed in the action of *B. atrosepticus*, *B. solanisaprus*, *B. melanogenes*, IIIA, SE, and IIP upon fresh skimmed milk sterilized by fractional steaming, except that SE produced a slow but evident digestion of the curd. With this exception noted the record for the group when grown at a temperature of 20° C. is: Coagulation somewhat delayed, not appearing for about 7 days; extrusion of whey does not take place until the end of 10 to 14 days, and only a slight amount is formed. Coagulum solid and apparently not digested, but readily breaks apart when probed with the needle, not slimy nor viscid, and when shaken forms nearly a solid mass with the whey. It will be noted that both Harrison (17) and Pethybridge and Murphy (26) report coagulation in 48 hours, but this is at 25° C. The latter gentlemen, however, recorded coagulation not appearing for five days at laboratory temperatures.

The two organisms carried under the name of *B. phytophthorus* were not tested upon milk and litmus milk.

LITMUS MILK.—On litmus milk the six organisms mentioned above produced at 20° C. a rather slow, moderate acid development at first, but later the cultures became strongly acid. At this temperature reduction or bleaching begins to appear at the end of one month's time. If the tubes were then heated sufficiently to kill the organisms, the red color promptly returned.

GELATIN COLONIES.—Colonies upon gelatin plate cultures made from meat-extract broth plus 10 per cent by weight of Nelson's photographic gelatin No. 1 and made neutral to phenolphthalein, incubated at 20° C. were identical in the case of *B. atrosepticus*, *B. solanisaprus*, *B. melanogenes*, and the three Maine organisms. These characters were: Growth rapid, buried colonies in 24 hours, round, margins entire. Under a 16-mm. objective and 12 eye-piece, the colonies at this time appeared nearly white and finely granular, usually with a faint indication of liquefaction at the margin. In 48 hours, in every case, a well-defined crateriform liquefaction had appeared and heavily seeded plates would be largely liquefied by this time at 20° C.

AGAR COLONIES.—The organisms mentioned in the preceding paragraph likewise gave characters identical with each other upon neutral meat extract agar. In 24 hours, viewed under a 16-mm. objective and 6 ocular, the colonies were brownish-yellowish in color, finely granular, margins entire, lens shaped to slightly ovoid or spherical. Viewed under a hand lens the colonies were slightly yellowish in color. The surface colonies were pearly white, bluish opalescent to transmitted light, flat, circular, and occasionally amœboid in shape if the plate were thinly seeded and the surface moist.

Some preliminary work was done, but no detailed studies were made with regard to the colonies produced upon gelatin and agar with the two organisms received and studied as *B. phytophthorus*.

FERMI'S SOLUTION.—In liquid cultures of Fermi's solution all the organisms showed a slight clouding in 24 hours. In 48 hours this was very well defined, but moderate, in the case of *B. atrosepticus*, *B. solanisaprus*, *B. melanogenes*, IIIA, SE, and IIP, with a tendency to form a pellicle, which in the case of SE was frequently quite well defined. *B. phytophthorus* from Appel and *B. phytophthorus* from Schuster at the same time had produced only a faint trace of cloudiness in the medium which later disappeared. Later observations showed considerable increase of cloudiness, almost to opaqueness in the case of the first six organisms named.

FERMI'S SOLUTION PLUS AGAR.—No attempt was made to grow the various organisms on Fermi's solution in silicate jelly, but one trial was made in which one and one-half per cent of agar was added and the sterilized tubes of media, slanted as in ordinary beef-peptone agar. On

these agar slants in 24 hours after being inoculated with a single stroke of a straight needle there was no visible growth. In 48 hours and later it was well defined and of uniform appearance in the case of *B. atrosepticus*, *B. solanisaprus*, *B. melanogenes*, IIIA, SE, and IIP. The character of the growth may be described as moderate, filiform, flat to slightly raised, smooth, glistening, plainly opalsecent, later opaque, color whitish or pearly, later creamy, odor absent, consistency butyrous—slightly viscid in the case of *B. solanisaprus* and *B. melanogenes*.

The two strains carried as *B. phytophthorus* produced only a very faint, semitransparent, filiform growth on this medium. No discoloration of the medium was observed in either case.

COHN'S SOLUTION.—No growth was obtained with any of the organisms when inoculated into Cohn's solution.

USCHINSKY'S SOLUTION.—The organisms received from Dr. Schuster as *B. phytophthorus* gave little evidence of growth in this medium, while all of the others produced a copious growth and behaved alike in it. Visible clouding with the latter was less during the first 24 hours than in broth tubes inoculated at the same time, but at the end of three days the medium was quite heavily clouded and milky in appearance.

SODIUM CHLORID IN BOUILLON.—One, two, three, four, and five gm. of sodium chlorid to each 100 c. c. of culture media were added to neutral beef infusion bouillon and narrow tubes each containing 5 c. c. of this salted medium were inoculated with a 2-mm. loop in the usual way.

At the end of 18 hours there appeared to be no inhibition of growth of any of the cultures in the tubes containing 1 and 2 per cent of sodium chlorid. In 3 per cent, all were slightly but plainly less clouded than the check tubes inoculated at the same time, and no growth had appeared in the 4 and 5 per cent. At the end of 48 hours all the tubes containing 3 per cent of sodium chlorid were apparently as well clouded as the checks. All showed growth in the presence of 4 per cent, but with some inhibition. In 5 per cent no growth was apparent in any of the tubes except those of *B. phytophthorus* from Appel, which showed about half-normal clouding for bouillon cultures of the same age. In three days *B. atrosepticus*, *B. solanisaprus*, *B. melanogenes*, IIIA, SE, and IIP in 5 per cent of sodium chlorid showed a faint but well-defined clouding for the first time and appeared quite uniform in appearance. *B. phytophthorus* from Appel was quite heavily clouded, while no growth could be detected in the culture of *B. phytophthorus* from Schuster.

GROWTH IN BOUILLON OVER CHLOROFORM.—This test was made with tubes containing 10 c. c. of sterile bouillon into each of which were introduced 5 c. c. of chloroform and the whole thoroughly agitated at intervals for two days. After allowing the tubes to incubate for several days to prove sterility they were inoculated with a 2-mm. loop of broth culture in the usual way, care being taken this time to agitate the medium

as little as possible and to introduce the inoculating loop only into the upper layers of the culture liquid.

In 18 hours there was no growth in any except *B. phytophthorus* from Schuster which showed a little clouding in the upper layers. Daily observations were made following this for several days.

In 36 to 48 hours as compared with normal broth cultures inoculated at the same time those of *B. atrosepticus*, *B. solanisaprus*, *B. melanogenes*, IIIA, SE, and IIP were much restrained, but there was an evident slight clouding all through, slightly stronger at the top. The same condition prevailed as long as the cultures were under observation, except that after three or four days the three organisms from Maine showed a more vigorous growth than the other three and produced a distinct, moderate clouding all through the bouillon. The appearance of the first three named was identical in all respects.

The growth in the cultures known as *B. phytophthorus* from Appel and from Schuster after about 48 hours was unrestrained and appeared equally as abundant as that in the check tubes.

BEST MEDIA FOR LONG-CONTINUED GROWTH.—Of the various media used, neutral beef bouillon, made either from meat infusion or from Liebig's extract, proved to be the best for long-continued growth.

PHYSICAL AND BIOCHEMICAL FEATURES

FERMENTATION OF CARBOHYDRATES.—The ability of the different organisms in this group to ferment various carbohydrates has furnished perhaps the most important differential characters upon which the previously named species have been erected. Therefore in the present studies more attention has been given to this subject than to any other. The results obtained are not based upon single trials, but upon repeated tests of each organism. All the fermentation tests were made in uniform fermentation tubes, having a small neck, a large bulb, and a capacity of about 25 c. c. in the closed arm. These were made to order and very closely conform to the illustration given by Smith (32, v. 1, p. 53). Both meat-infusion bouillon, previously freed from muscle sugar by inoculation with *B. coli*, and meat-extract bouillon made from Liebig's extract and tested for the absence of fermentable carbohydrates, as previously described, were used. The meat-extract bouillon was used for a large proportion of the work, for it was found to be more satisfactory. For the fermentation work 1 per cent of the carbohydrate used was added to the culture medium and the tubes containing the media were sterilized by fractional steaming. The following substances were tested for fermentation in this way: Dextrose, saccharose, lactose, maltose, glycerin, mannit, and dextrin. In addition some of the organisms were tested in the neutral-red lactose broth which is commonly used in water work as one of the presumptive tests for *B. coli*. The culture medium in each case was made neutral to phenolphthalein.

To save unnecessary repetition it may be stated in the beginning that the two organisms received and carried under the name of *B. phytophthorus* produced no gas or acid in the presence of any of these substances and imparted a slight alkalinity to the culture medium. The one received from Dr. Appel in no case produced any visible clouding of the closed arm of the fermentation tube. The one received from Dr. Schuster produced a delayed clouding of the closed arm in all cases except with glycerin, but even with this the clouding was later nearly as complete as in the bulb.

The results obtained with the remaining organisms—namely, *B. atrosepticus*, *B. solanisaprus*, *B. melanogenes*, IIIA, SE, IIP—were alike and constant with regard to gas formation, growth in the closed arm, and production of acid. On repeated trials they all invariably produced gas in the presence of dextrose, saccharose, lactose, maltose, and mannit. They produced no gas from glycerin and dextrin. With dextrose this amount of gas was small, ranging from 3 to 5 per cent. With saccharose the percentage of gas was higher, usually being from 7 to 9 per cent. Except with glycerin, there was always a prompt clouding of the closed arm, though this was usually less than in the bulb. This clouding in the closed arm was persistent, although a slight clearing at the top of the tube was observed in old cultures containing maltose. Acid was produced in the presence of all the carbohydrates used.

Very little of the gas which was formed was absorbed by a 2 per cent solution of sodium hydrate. The remainder of the gas was explosive. When expressed in the terms of $H:CO_2$, as accurately as could be determined, this varied from 1:0 to 5 or 6:1. No attempt was made to determine further the nature of this gas. Gas in all cases usually appeared on the second or third day and reached its maximum before the tenth, usually on the sixth or seventh.

It will be noted that this constant appearance of gas in dextrose, saccharose, and maltose with *B. solanisaprus* was contrary to Harrison's original description (17). Likewise the production of gas with *B. atrosepticus* from lactose and dextrose differs from the results recorded by Van Hall. In gas production *B. melanogenes* agreed in every respect to Pethybridge and Murphy's description (26).

In neutral-red lactose fermentation broth the amount of gas obtained in each case with the 6 different organisms was the same as with the ordinary lactose broth. In 48 hours the entire contents of the tube appeared distinctly more red than the check. On the third day the closed arm took on a yellowish olive tinge, which later changed to a canary-yellow, stronger even than that produced by *B. coli*, run at the same time for comparison. This color persisted for one month, or as long as the cultures were under observation. During the same time the bulb showed a more pronounced red than the check tube.

AMMONIA PRODUCTION.—Cultures in Dunham's peptone solution were tested at the end of one, two, three, and four days as follows: In clean test tubes of the same internal diameter there were placed 10 c. c. of ammonia-free water. Into each was placed one 2-mm. loopful of the culture to be tested, taken from the top of the tube, and then six drops of Nessler's solution was added to each tube of water and culture dilution. The color of the tubes was observed by looking vertically through them upon a white background. No color developed in any of the tubes except in those containing some of the culture of *B. phytophthorus* from Schuster. The latter gave a distinct yellow color.

Pethybridge and Murphy (26) have recorded the appearance of small amounts of gas in cultures of *B. melanogenes* in tubes of a 2 per cent potassium-nitrate broth having a plug of vaseline on the top. For the purpose of testing all of the organisms in this respect, fermentation tubes were filled with the nitrate broth described in the next section, sterilized by fractional steaming, and then inoculated. Growth appeared in these rather slowly but at the end of 48 hours all were uniformly though somewhat faintly clouded with the exception of the two strains carried under the name of *B. phytophthorus*. These showed only growth in the bulb. Later they showed some clouding of the closed arm of the tube, but this was exceedingly faint in the case of *B. phytophthorus* from Appel. No gas whatever appeared in the closed arm of the tubes with any of the organisms studied. Tubes of this same broth, tested at the end of five days, with Nessler's reagent by dropping five or six drops of the reagent directly into the tubes gave no qualitative test for ammonia, except in the case of *B. phytophthorus* from Schuster. Cultures of the latter when treated with the reagent produced a very distinct, yellow reaction. That the Nessler's solution used was of good quality was indicated by the fact that it would produce the characteristic reaction in the presence of very minute quantities of ammonia, artificially introduced into distilled water.

In connection with these tests it should be noted that Pethybridge and Murphy used a 2 per cent potassium-nitrate broth, while the writer used that specified by the Committee on Standard Methods of Water Analysis,[1] which carries only 2 gm. of potassium nitrate per liter.

Where Nessler's solution was added directly to young cultures in Dunham's peptone solution, in no case was there any more color observed to result than where the uninoculated check tube of the same medium was so treated, except in the case of *B. phytophthorus* from Schuster. Cultures of this organism gave a yellow color reaction.

REDUCTION OF NITRATES.—Cultures in nitrate broth, consisting of 2 gm. of chemically pure potassium nitrate, 1 gm. of Witte's peptone and 1 liter of water, were tested by the starch-iodin method after five days. All

[1] Report of committee on standard methods of water analysis to the laboratory section of the American Public Health Association, presented at the Havana meeting, January 9, 1905. 141 p. Chicago, Ill., 1905. Reprinted from Jour. Infect. Diseases, suppl. 1, 1905.

except *B. phytophthorus* from Appel when so tested at once showed a deep-blue color reaction, indicating the presence of nitrites. No such color appeared in the uninoculated check tubes of the same medium tested at the same time.

From what is said in the preceding section it might be inferred that *B. phytophthorus* from Schuster is apparently able to reduce nitrates to nitrites and then to ammonia. However, it should be remembered that the nitrate broth used contained a small quantity of peptone, and it has been shown that the organism is able to produce ammonia from peptone. Doubtless this was the source of the ammonia reaction in the nitrate broth.

INDOL PRODUCTION.—Cultures of *B. atrosepticus*, *B. solanisaprus*, *B. melanogenes*, the three Maine organisms and of the one received from Schuster as *B. phytophthorus* gave a positive reaction to the indol test. That received from Appel as *B. phytophthorus* and the check tubes always gave a negative reaction. The pink color, while well defined, and sometimes in old cultures being quite marked, was very much less than that produced by *B. coli*, which was tested at the same time for comparison. In the case of the positive-reacting organisms the color was very slight or absent in cultures four to seven days old and was most marked in cultures two months old or more. Therefore, indol production where it occurred would be classed as being feeble to moderate.

TOLERATION OF ACIDS.—In the present studies only hydrochloric acid has been tested. Normal hydrochloric acid was added to meat-extract broth which in the beginning was neutral to phenolphthalein, in sufficient quantities to make it +10, +20, +30, +40, and +50 Fuller's scale.

In from 12 to 18 hours all of the organisms showed equally good growth in the check tubes of neutral broth. At +10 the amount of clouding was nearly equal to that produced in the checks. At this time there was a marked falling off in the clouding at +20 as compared with the check tubes, and no growth had appeared in the presence of the larger amounts of acid.

At 48 hours and later there was no change in the relative amounts of cloudiness exhibited by the different organisms, except that cultures of *B. phytophthorus* from Appel were slightly less cloudy and *B. phytophthorus* from Schuster were slightly more cloudy at +20 than those of *B. atrosepticus*, *B. solanisaprus*, *B. melanogenes*, IIIA, SE, and IIP. The latter were all alike in their behavior toward hydrochloric acid. None of the various organisms produced any growth at +30 or beyond.

TOLERATION OF SODIUM HYDRATES.—Cultures of all of the organisms were made in meat-extract bouillon, neutral to phenolphthalein, in comparison with the same medium made −10, −20, −30, −40, and −50 Fuller's scale with normal sodium-hydrate solution.

At the end of 12 to 18 hours the cultures in the −10 bouillon were as heavily clouded as the neutral; those at −20 were well clouded but plainly less than at −10. At this time *B. atrosepticus*, *B. solanisaprus*, *B. melanogenes*, IIIA, SE, and IIP were practically identical in the amount of cloudiness they produced on the different media, but showed no growth beyond −20. *B. phytophthorus* from Appel and *B. phytophthorus* from Schuster showed a decreasing amount of cloudiness up to and including −40, but they showed no growth in the tubes of media with a reaction of −50.

In 48 hours the last two named had produced a faint but evident growth in the medium having a reaction of −30. At the end of two weeks all showed growth at −40 and all but *B. atrosepticus* and SE at −50.

Optimum reaction for growth in bouillon.—This is approximately the same for all of the organisms studied. It lies very close to the neutral point of phenolphthalein, but is apparently on the alkaline side. Observations to determine this must be made with these organisms during the first 12 to 18 hours of growth. Broth cultures with a reaction of +10 with hydrochloric acid and −10 with sodium hydrate, Fuller's scale, were practically equally well clouded in 24 hours, but the acid cultures in the early stages of growth were observed to cloud slightly more slowly. Sodium hydrate restrains the growth much less than does hydrochloric acid, but in both alkaline and acid media of stronger reaction than that first mentioned there was a very decided falling off in the clouding during the first 24 hours as compared with that obtained with media of neutral reaction.

Vitality on culture media.—Cultures of *B. solanisaprus* and the three organisms from Maine in neutral beef-extract bouillon, stored at a temperature of from 15° to 18° C., were found to be alive at the end of 8 to 10 months, provided the moisture had not entirely evaporated. Tests were made with the other organisms, but with somewhat younger cultures and in each case they were found to be alive in the bouillon. Cultures in liquefied gelatin were also found to possess long vitality, but upon agar slants, milk, and the various carbohydrate broths, the organisms were killed out much more readily. Those in milk were frequently found to be entirely dead at the end of three months.

Temperature relations.—The optimum temperature for growth in the six pathogens studied is not far from 25° C., although no attempt was made to determine this within a variation of 5° C. above and below. Numerous tests were made of all the strains at the same time in seven different incubator chambers, running from 5° to 35° C. Considerable differences were noted in the rates of growth at lower temperatures, even in different tests of the same strain, but as the optimum was approached a striking uniformity was obtained. This is well illustrated by Table III, which gives the results secured from a single series. In the temperature

columns of this table are given the number of hours which elapsed from the time a 10-c. c. broth culture was inoculated with a 2-mm. loop of a 24-hour-old broth culture, before the first visible traces of clouding of the medium could be observed, observations being made hourly. In all cases the tubes of media were placed in the incubator at the desired temperatures for a few hours before inoculation, and care was taken not to remove them therefrom any longer time than was necessary to make the inoculations and observations.

TABLE III.—*Effect of temperature on rate of growth of the organisms*

Organism.	First evidence of clouding of broth cultures at—						
	5° C.	10° C.	15° C.	20° C.	25° C.	30° C.	35° C.
	Hours.	*Hours.*	*Hours.*	*Hours.*	*Hours.*	*Hours.*	*Hours.*
B. atrosepticus	84	27	22	13	11	12	No growth.
B. solanisaprus	96	27	22	13	11	12	Do.
B. melanogenes	52	27	22	13	11	12	Do.
IIIA	84	28	21	14	11	12	Do.
SE	96	27	21	16	11	12	Do.
IIP	192	59	27	16	13	16	Do.
B. phytophthorus from Appel.	192	64	30	18	13	12	16.
B. phytophthorus from Schuster.	192	60	22	13	8	11	12.

It will be seen that while the optimum for the two nonpathogenic strains carried under the name of *B. phytophthorus* is about the same temperature as for the six pathogens, the former grew at 35° C., while the latter did not.

Both agar slants and beef-broth cultures were used to determine the maximum temperature for growth in the case of *B. atrosepticus*, *B. solanisaprus*, *B. melanogenes*, and the three pathogenic strains isolated in Maine. On beef-extract broth neutral to phenolphthalein this was not far from 33° C.; if anything, slightly below this. None of the six produced visible clouding of broth at 34° C. and above. On agar slants the growth was scanty or absent at 32° and 32.5°, *B. atrosepticus*, SE, and IIP giving the most frequent failures to produce growth on agar at these temperatures. All of the pathogenic strains would show very evident growth in broth at 32° in 18 hours, and also clouding in the same period of time, with less regularity and with some failures to produce clouding at all at 32.5°. Undoubtedly the somewhat lower maximum temperature for growth on agar was due to the rapid drying out of the surface of the medium. The results given above were checked a number of times, since they were unexpected on account of Harrison having reported growth with *B. solanisaprus* at 37°.

The thermal death point for *B. atrosepticus*, *B. solanisaprus*, *B. melanogenes* and the three pathogenic strains isolated from Maine was found to be approximately 46.5° C. Retests at this temperature did not

always prevent later clouding of the inoculated and heated tubes of broth. In no case, except with a single tube of *B. melanogenes*, did growth occur after heating at 47° and above, but clouding always appeared after an exposure to 46° C. and below. Transfers from well-clouded broth cultures 36 to 48 hours old appeared more resistant than those a few days older. The strains carried under the name of *B. phytophthorus* showed a much higher thermal death point. For the culture received from Dr. Appel it was between 51° and 52°, while that from Dr. Schuster was killed at 54° but not at 53°.

In making the thermal-death-point determinations thin-walled test tubes of 16 to 17 mm. internal diameter and approximately 16 cm. long were employed. Each tube contained 10 c. c. of meat-extract broth, +15 Fuller's scale.

Immediately after inoculation they were transferred to a specially constructed water bath, provided with a stirring apparatus and an accurate, certified thermometer, and immersed nearly their entire length in the heated, constantly moving water. The period of immersion was 10 minutes and the bath during this time was maintained within one-tenth of 1 degree C. of the required temperature.

EFFECTS OF DRYING.—The effect of drying was tested as follows: A 2-mm. loop of a 24-hour broth culture was removed to and spread upon small, sterilized, cover glasses in sterile petri dishes. These were allowed to dry at the same temperature as incubation for varying periods after the last trace of moisture had disappeared from the cover glasses. Then the latter were picked up with flamed forceps and dropped into tubes of sterile bouillon. Only *B. solanisaprus*, IIIA, and SE were tested in this way. The results were somewhat variable. Usually one or two minutes' drying were sufficient to produce sterility, although in one or two cases growth appeared after 10 and 15 minutes' drying. However, in each case like this the growth was much retarded.

EFFECT OF SUNLIGHT.—The effect of sunlight was tested only in the case of the three organisms mentioned in the preceding paragraph. The usual method was followed: The freshly inoculated and half-covered petri-dish cultures were exposed on blocks of ice to bright sunlight at midday in a greenhouse. There was no diminution of resulting colonies on the exposed side in 10-and 20-minute exposures, slight diminution at 30 minutes, while at 60 minutes all the organisms on the exposed side were killed, and no colonies developed. This test was made in November and consequently the conditions were not favorable. Doubtless the same exposure outdoors in midsummer would have shown sunlight to be more effective in killing the organisms.

EFFECT OF GERMICIDES.—In testing the effect of germicides, only mercuric chlorid and formaldehyde were employed, and only *B. solanisaprus*, IIIA, and SE were tested. The results were the same with

all three organisms. In the case of the mercuric-chlorid test one 2-mm. loop of a deeply clouded, 24-hour broth culture was transferred to check tubes of sterile distilled water and to others containing varying dilutions of the poison, the weakest being 1 to 50,000. After one hour transfers were made from these to fresh broth tubes. In no case was growth obtained except from the inoculated check tubes of pure water. Where mercuric chlorid was added directly to broth tubes it was found that it required a concentration of 1 to 10,000 to produce the same results.

With formaldehyde no attempt was made to determine how weak a solution would be effective. It was simply tested and found that one part in 250 in distilled water or beef broth was sufficient to kill all organisms in an hour's exposure. This was somewhat weaker than is usually used for disinfecting potato tubers and the time one-half as long.

PATHOGENICITY

As has already been stated, all of the strains studied were tested at various times to determine their ability to produce the characteristic blackleg disease of the stem and softrot of the tuber. From the beginning to the close of the work *B. solanisaprus*, *B. melanogenes*, and the three strains from Maine were found to be pathogenic to potato stems, leaf stalks, and tubers.

When first received in March, 1910, the culture of *B.atrosepticus* either failed to attack or caused a slow decay of pieces of potato tubers produced the season before, which had been cut under aseptic conditions and placed in tubes of sterile water. However, after several repeated transfers from one 24-hour-old potato-broth culture to another it was found to produce a fairly rapid decay when inoculated into potato tubers, especially those which were immature and recently dug. This strain has continued to rot tubers, provided they were not too mature and care was taken not to allow the inoculated portion to dry out too rapidly..

It has been the custom whenever the pathogens were tested on growing stems of potato plants to inoculate a plant each time with each of the nonpathogenic strains. *B. atrosepticus* was repeatedly used in this way, but the results were negative previous to June 15, 1916. A plant inoculated on this date by puncturing the young, growing stem near the base with the needle of a hypodermic syringe and inserting a small amount of a 24-hour-old culture in the tissues of the pith, was observed five days later to be showing slight blackening around the point of inoculation. Closer examination showed that the pith had been softened and decayed for a short distance above the puncture. This diseased condition did not progress much farther, however. About a month later several other young stems were inoculated with transfers of the stock strain of *B. atrosepticus* and this time the positive results were more marked. The inoculated stems were blackened on the surface for a dis-

tance of 3 to 4 inches and finally fully decayed and cut off, resulting in the death of that part of the plants above the points of inoculation.[1]

As has been clearly indicated in the previous pages, inoculations of potato stems and tubers with the cultures received and carried under the name of *B. phytophthorus* produced no disease whatever. The punctures into which the cultures were inserted dried out rapidly and were in every way similar to those into which sterile broth or distilled water had been inserted as checks.

Some work was done to test the pathogenicity of the strains used, upon plants closely related to and unrelated to the potato. It will be unnecessary to go into the details of this since the present studies are primarily concerned with a comparison of the various morphological, cultural, physical, and biochemical features of the different organisms.

GROUP NUMBERS

The numerical system employed in the descriptive chart or card adopted by the Society of American Bacteriologists furnishes a means whereby a considerable number of contrasted, salient characters may be brought together in a small space to facilitate comparisons. This decimal system of group numbers is useful as a quick method of showing close relationships and important differences within the genus, but is not a sufficient characterization for any organism. However, it does record in a very compact manner many of the important differential characters.

GROUP NUMBERS BASED ON THE PRESENT STUDIES

B. atrosepticus Van Hall	221. 1113522
B. solanisaprus Harrison	221. 1113522
B. melanogenes Pethybridge and Murphy	221. 1113522
IIIA	221. 1113522
SE	221. 1113522
IIP	221. 1113522
B. phytophthorus (as received from Appel)	222. 3333033
B. phytophthorus (as received from Schuster)	221. 3333533

Some question might be raised with regard to whether the figure in the fifth place to the right of the decimal point in the first six group numbers should be a 5 or a 0. Here the only yellow color that developed was on potato, and this was by no means strong. It is possible also that the next figure to the right in the same group numbers should be 3, for the diastatic action on potato starch is doubtful.

[1] The writer has no explanation to offer for this unexpected manifestation of pathogenicity on the part of this culture of *B. atrosepticus*. The system used in keeping and transferring the stock cultures is such that any possibility of getting the different strains mixed is practically eliminated. The fact that the stock cultures have always been carried in beef broth with occasional transfers to potato broth may have something to do with it. Others who have kept their stock cultures of these and the closely related softrot bacteria of the *B. carotovorus* type on agar have reported loss in pathogenicity. Including those mentioned in this paper the writer has carried over 20 different strains of blackleg organisms for from 5 to 9 years, but with no permanent evidence of loss of pathogenicity with any.

In the case of the organism received from Schuster and carried under the name of *B. phytophthorus*, the yellow growth was apparent on both agar and potato. Upon the latter the growth was of a very distinct lemon yellow, considerably deeper than that produced by the other organisms.

NOMENCLATURE AND RELATIONSHIP

An analysis of the data obtained as the result of these comparative studies seems to point to but one conclusion. When subjected to the various differential tests at the same time and under the same conditions, the cultures received under the names "*Bacillus atrosepticus* Van Hall," "*Bacillus solanisaprus* Harrison," and "*Bacillus melanogenes* Pethybridge and Murphy" and the three strains of organisms isolated from potato plants affected with the blackleg disease from widely separated parts of Maine appear to be identical.

It is the writer's opinion that the pathogenic organisms studied should be classed as one species, or at the most strains of the same species. The only constant differences noted were slight variations in size, shown more particularly by *B. solanisaprus* and IIIA, and the production of a slight viscidity on different kinds of media shown by *B. solanisaprus* and *B. melanogenes*, but not by any of the others.

It is evident that the two organisms which the writer received and studied under the name of *Bacillus phytophthorus* Appel were not alike, nor were they the same as the one originally described by Dr. Appel.

There is nothing in the data here presented which bears on the relationship between the organism originally described by Dr. Appel as *B. phytophthorus* and the other strains of blackleg bacteria. As has already been stated, Dr. Smith states that it and *B. solanisaprus* are not identical, but are closely related. While the writer regrets that he was unable to get an authentic, virulent culture of this organism for comparison in time to make use of it he has no reason for questioning this statement, coming from so good an authority.

While the present studies are primarily concerned with relationship, the results obtained make it impossible to entirely ignore the question of nomenclature. If it is granted that *B. phytophthorus* differs from *B. solanisaprus* and consequently from the others under consideration, which of the three other names should be retained? On grounds of priority, *B. melanogenes* is excluded and the choice is between *B. atrosepticus* and *B. solanisaprus*. Also on the grounds of priority alone it would seem that the accepted name should be *B. atrosepticus*, but for certain reasons the writer was at first inclined to favor *B. solanisaprus*. These reasons are as follows: According to present standards *B. atrosepticus* was not very fully described in the beginning. Moreover, the culture used by the writer was obtained from Kral's laboratory with no statement regarding its origin or authenticity. Also its pathogenicity was erratic or weak,

and for a long time it entirely failed to produce typical blackleg of the stem upon inoculation. This was in accord with the impression one would gain as to Van Hall's results relative to pathogenicity on reading certain reviews of his paper (15). When Dr. De Zeeuw's translation of Van Hall's paper became available, the results of his inoculation experiments appeared in quite a different light. These were quite limited, on account of a lack of a sufficient amount of proper material. As is the case with any of the pathogenic strains, the inoculation experiments with mature stems and tubers gave erratic results or failures. He was able in some instances to produce typical blackening and decay when young parts of stems were inoculated.

B. solanisaprus, on the other hand, was originally described in much detail, and authentic, virulent cultures are to be obtained by anyone who may wish to study it. That used by the writer is as pathogenic to-day to potato stems and tubers as when described by Harrison 10 years ago.

In recommending the dropping of the name "*B. melanogenes*," the writer does not wish to be understood as casting any reflections upon the work of Pethybridge and Murphy, or upon their conclusions, based upon the results obtained by them when compared with published descriptions. The data obtained in the present studies have checked in every essential detail with their description of this organism except that the writer secured a positive test for indol and was unable to get the formation of gas in nitrate broth. However, attention has already been called to the fact that Pethybridge and Murphy used a broth containing a much greater percentage of potassium nitrate than was used by the writer. It should be noted that the agreement between *B. solanisaprus* and *B. melanogenes* resulted from the writer's obtaining certain results, especially with the fermentation of the carbohydrates and the liquefaction of gelatin, which were different from those reported by Harrison. Also Harrison reports the thermal death point for *B. solanisaprus* to be 54° C., while Pethybridge and Murphy say that for *B. melanogenes* it lies between 45° and 50°. The writer's results showing that the thermal death point is approximately 46.5° are then in accord with the latter statement.

REVISED DESCRIPTION OF BACILLUS ATROSEPTICUS VAN HALL, GROUP NUMBER 221.1113522 [1]

I. MORPHOLOGY

Vegetative cells. Medium used was an agar slope at 20° C. 1 to 2 days old. Form, short rods, long rods, short chains, long chains. Limits of size in microns (stained preparations) 0.4 to 0.8 by 1 to 2 or more. Size of majority 0.5 to 0.6 by 1.5 to 2. Ends rounded.

Endospores, none.

Flagella, few, not over 6 or 8. Attachment, peritrichiate. Stained by modified Pitfield method.

[1] See statement following the list of group numbers on p. 120.

Capsules, none.

Pseudozoogloea, present, slight. Involution forms, not observed. (Reported by Harrison for *B. solanisaprus*, at higher temperatures.)

Staining reactions. Stains well in aqueous gentian violet, anilin water gentian violet, aqueous methylene blue, alkaline methylene blue, aqueous fuchsin, carbol fuchsin. Gram, negative.

II. CULTURAL FEATURES

Agar stroke. Growth, moderate. Form of growth, filiform. Elevation, flat to slightly raised. Luster, glistening. Topography, smooth. Optical characters, slightly but distinctly bluish opalescent. Chromogenesis, pearly white. Odor, absent. Consistency, butyrous—some strains slightly viscid, others not.

Potato. Growth, moderate to abundant. Form of growth, filiform to spreading. Elevation, first convex, then slightly raised to flat. Luster, glistening. Topography, smooth to slightly rugose. Chromogenesis, yellowish white, later dirty white. Slight odor of decayed potatoes in old cultures. Consistency, butyrous—some strains slightly viscid, others not. Medium, slightly grayed at first, later plainly grayed.

Agar stab. Growth, slightly best at top, abundant, widespreading. Line of puncture, filiform to slightly papillate.

Gelatin stab. Growth, best at top. Line of puncture, filiform. Liquefaction, infundibuliform to slightly napiform, later may be saccate, occasionally crateriform to stratiform. Begins in 1 to 3 days at 20° C. Complete in 7 to 10 days with some cultures, in others not complete in 60 days.

Nutrient broth. Surface growth, usually slight ring and occasionally slight granular pellicle in young cultures. Clouding, moderate to strong, persistent. Medium, not discolored. Odor, absent. Sediment, compact, scant, granular; and dirty white—some strains slightly viscid, others not.

Milk. Coagulation, usually not until the seventh day at 20° C. Coagulum, not digested or at the most very slowly peptonized. Medium, not discolored.

Litmus milk. Acid, litmus reduced.

Gelatin colonies. Growth rapid at 20° C. Form, round. Edge, entire. Liquefaction, saucer.

Agar colonies. Growth, rapid at 20° C. Form, round, occasionally irregular, buried colonies, lens-shaped to slightly ovoid or spherical. Surface, smooth. Elevation, flat to slightly raised. Edge, buried colonies, entire, surface colonies, entire to undulate. Internal structure, finely granular. Color buried colonies, brownish yellow under a 16 mm. objective and 6 ocular, slightly yellowish under hand lens, surface colonies, pearly white, bluish opalescent to transmitted light.

Fermi's solution. Growth, first moderate, later abundant.

Cohn's solution. Growth, absent.

Uschinsky's solution. Growth, copious.

Sodium chloride in bouillon. Growth inhibited, 3 per cent, slightly, 4 per cent, some, and 5 per cent, considerably.

Growth in bouillon over chloroform. Growth, much restrained at first, later moderate.

Best medium for long-continued growth. Neutral beef bouillon.

III. PHYSICAL AND BIOCHEMICAL FEATURES

Fermentation tubes. Gas produced from dextrose, saccharose, lactose, maltose, and mannit, but not in glycerin and dextrin. Growth in the closed arm with dextrose, saccharose lactose, maltose, mannit, and dextrin, but either absent or slight with glycerin. Acid produced from dextrose, saccharose, lactose, maltose, mannit, and glycerin.

Ammonia production, absent.

Nitrates in nitrate broth reduced to nitrites.

Indol production, moderate in old cultures, absent or feeble in young cultures.

Toleration of acids. Grows in broth at +20 Fuller's scale and not at +30 with hydrochloric acid.

Toleration of sodium hydrate, great.

Optimum reaction for growth in bouillon in terms of Fuller's scale, 0 or slightly alkaline.

Vitality on culture media. Relatively brief on agar and media containing carbohydrates; long on bouillon.

Temperature relations. Thermal death point, approximately 46.5° C. Optimum temperature for growth, about 25° C. Maximum temperature for growth, about 33° C. Minimum temperature for growth, below 5° C.

Effects of drying. Readily killed.

Exposure to sunlight. Exposure on ice at midday in November, slight diminution at 30 minutes, 100 per cent killed at 60 minutes.

IV. PATHOGENICITY

Virulent strains produce rapid decay accompanied by a pronounced blackening of the surface tissues when inoculated into young, growing potato stems; also causes a rapid softrot of potato tubers at moderate temperatures and may produce decay when inoculated into a considerable number of fleshy vegetables.

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